Analysis of silicone oil in tall oil products

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TABLE OF CONTENTS

ABBREVIATIONS

BSTFA	bis(trimethylsilyl)trifluoroacetamide
CH17	cholesteryl heptadecanoate
CTO	crude tall oil
FTIR	Fourier transform infrared spectroscopy
GC	gas chromatography
GC-FID	gas chromatography flame ionization detector
GC-MS	gas chromatography mass spectrometry
GPC	gel permeation chromatography
HPSEC	high-performance size-exclusion chromatography
HVO	hydrotreated vegetable oil
ICP-MS	inductively-coupled plasma mass spectrometry
LT-ELSD	low-temperature evaporative light-scattering detector
MTBE	methyl *tert*-butyl ether
MW	molecular weight
NMR	nuclear magnetic resonance spectroscopy
PDMS	polydimethylsiloxane
PFTE	polytetrafluoroethylene
SPE	solid-phase extraction
TG	triglyceride
THF	tetrahydrofuran
TLC	thin-layer chromatography
TMCS	trimethylchlorosilane
TOP	tall oil pitch

1. INTRODUCTION

Biorefining is the sustainable conversion of biomass feedstock into marketable bioproducts and bioenergy such as transportation biofuels, power and heat, biomaterials, and value-added chemicals [1]. In recent years, underutilised process streams and waste materials have been tapped and developed to create more viable options for our increasing energy requirements. Biorefineries are building capabilities to process forest-based biomass and mill residues from the pulp and paper industry to produce road transportation biofuels [2].

Tall oil is one of the value-added by-products of the kraft or sulfate pulping process which is composed of a mixture of fatty acids, resin acids, and unsaponifiable neutral compounds [3,4]. Fatty acids (e.g., oleic acid, linoleic acid, stearic acid, and palmitic acid) are linear, long-chain carboxylic acids which consist primarily of C_{18} (18 carbon atoms) chains with small amounts of C_{16} and C_{20} chains [3,5]. The most common fatty acids found in tall oil are shown in Figure 1.

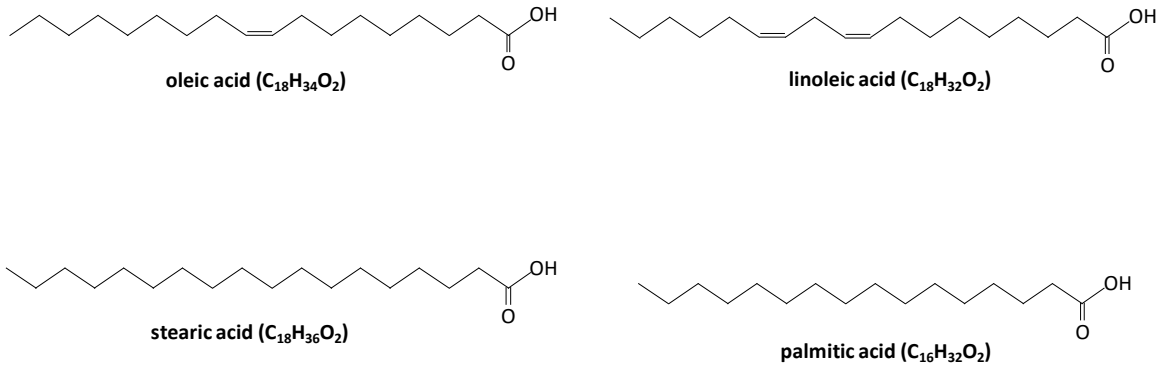


Figure 1. *Chemical structures of common fatty acids (oleic, linoleic, stearic, and palmitic acids).*

Resin acids are tricyclic diterpenoids with an empirical formula $C_{20}H_{30}O_2$, and are classified into two types: abietane, and pimarane types (Figure 2). Abietane type has an isopropyl or isopropenyl group attached to C-13 while pimarane type has vinyl and methyl groups [6].

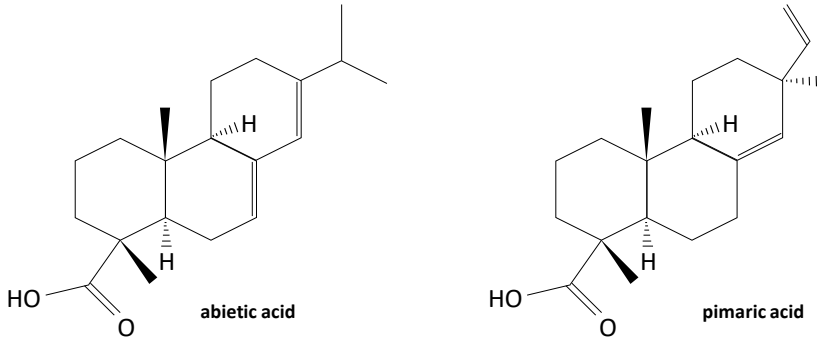


Figure 2. *Chemical structures of tricyclic diterpenoids abietic and pimaric acids.*

The unsaponifiables (e.g., polycyclic hydrocarbons, high molecular weight (MW) fatty alcohols, and sterols) are neutral compounds that do not form salts upon addition of a strong alkali such as sodium hydroxide (NaOH) [3,4]. Steryl esters, presented in Figure 3, are formed when sterols and fatty acids undergo esterification [6].

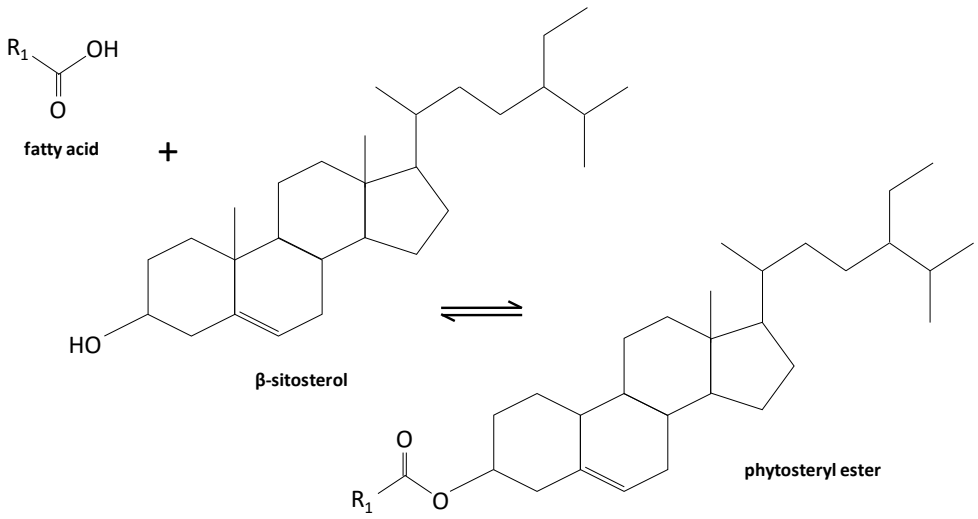


Figure 3. *Esterification of β-sitosterol and a fatty acid to phytosteryl ester.*

Crude tall oil (CTO) is formed by skimming off tall oil soap or the top layer of concentrated black liquor and reacting the ionic soap with concentrated sulfuric acid to form carboxylic acids, following the reaction [3,4,5]

$$R\text{—}COO^{-}Na^{+} + H^{+} \rightarrow R\text{—}COOH + Na^{+} \qquad (1)$$

The composition of a typical pine CTO is 50% fatty acids, 40% resin acids, and 10% neutral compounds [6]. CTO can be refined by fractional distillation to produce five tall oil products with different boiling points: heads (light ends), fatty acids, distilled tall oil (a mixture of fatty and resin acids), resin acids, and pitch or residue [3,4,5].

Tall oil pitch (TOP) is a non-volatile residue of crude tall oil distillation, with 15-40% yield from the refining process [7]. Although it is considered as a non-volatile component of CTO, it still contains volatile components with relatively low vapour pressures [3]. TOP is used in applications for alkyd-type coating resins, asphalt additives, printing inks, rubber compounding, and as a low sulfur fuel [3,5]

In papermaking processes, antifoaming agents are used in controlling or reducing foam problems [8] that disturb the washing efficiency and other operating conditions. Antifoams are generally classified into two types: organic antifoams or silicone antifoams. Organic antifoams are formulated based on mineral, paraffin, or vegetable oils, combined with particles from amide waxes or hydrophobised silica while silicone antifoams are formulated based on polydimethylsiloxane (PDMS) fluids with hydrophobised silica particles, combined with silicone polyethers or hydrophilic organic polyethers to enhance the emulsification of the silicone compound [9].

PDMS, the active component in silicone antifoam compounds, has very low surface energy [10]. It is non-volatile, hydrophobic with limited solubility in water, and is the most common organosilicon polymer used in coatings, personal care products, detergents, heat transfer fluids, dielectric fluids, and antifoams [10]. PDMS can be classified according to average MW: low-molecular (up to 10 000 Daltons or 10 kDa), intermediate-molecular (10-30 kDa), and high-molecular (>30 kDa) [8]. The chemical structure of PDMS, with its repeating monomer –[$(Me)_2$Si–O–] unit, is shown in Figure 4 [11].

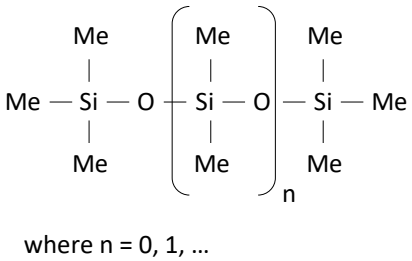


Figure 4. *Chemical structure of a trimethylsilyloxy terminated polydimethyl siloxane (PDMS).*

High MW PDMS is generated from the hydrolysis of dimethyldichlorosilane (Me_2SiCl_2) to linear and cyclic oligomers (Eq. 2), and the polymerisation of cyclic oligomers in the presence of an end-blocker such as hexamethyldisiloxane ($Me_3SiOSiMe_3$) using a strong acid or base as catalyst (Eq. 3) [11].

$$x\ Me_2SiCl_2 \xrightarrow[-\ HCl]{+\ H_2O} x\ \underset{\textbf{\textit{disilanol}}}{\text{“}Me_2Si(OH)_2\text{”}} \xrightarrow{-\ H_2O} y\ \underset{\substack{\textbf{\textit{linear}}\\ \textbf{n = 20-50}}}{HO(Me_2SiO)_nH} + z\ \underset{\substack{\textbf{\textit{cyclic}}\\ \textbf{m = 3, 4, 5, … (mainly 4)}}}{(Me_2SiO)_m} \quad (2)$$

$$Me_3SiOSiMe_3 + x\ (Me_2SiO)_4 \xrightarrow{cat.} Me_3SiO(Me_2SiO)_nSiMe_3 \quad (3)$$

Graiver et al. [10] studied different degradation mechanisms of PDMS. Upon incineration, high MW PDMS depolymerises to low MW volatile organosiloxanes that are eventually degraded by hydroxyl radicals (OH·). Depolymerisation also occurs in soil catalysed hydrolytic degradation by cleaving the siloxane bonds to produce low MW silanol terminated oligomers: dimethylsilanediol and trimethylsilanediol (Figure 5). The low MW silanols are sufficiently volatile and are degraded by hydroxyl radicals in the upper atmosphere by cleaving the silicon-carbon (Si-C) bond to yield silica, water, and carbon dioxide.

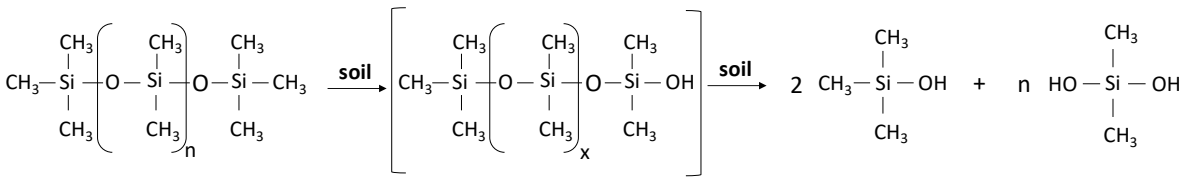


Figure 5. *Soil catalysed hydrolytic degradation of PDMS to dimethylsilanediol and trimethylsilanediol.*

Silicone antifoams are commonly delivered as water-based emulsions, and the high MW PDMS antifoams are formulated to be more resistant towards deactivation [9]. With this improved formulation for silicone antifoams, significant traces of PDMS may still be present in the tall oil products, either as high MW PDMS or as linear and cyclic oligomers. These siliceous components are suspected to have a negative effect on the performance of hydrotreating catalysts in the oil refining industry [12].

Preliminary inductively-coupled plasma mass spectrometry (ICP-MS) analysis of TOP samples conducted by an external laboratory resulted in total elemental silicon (Si) concentrations ranging from 10 to 100 ppm. However, at such low concentrations, several analytical methods such as nuclear magnetic resonance (NMR) spectroscopy and Fourier transform infrared (FTIR) spectroscopy cannot directly measure the high MW PDMS components (typically the detection limit is 1%). Therefore, a series of preconcentration steps are necessary to obtain a fraction that contains the high MW PDMS through different separation techniques to overcome the limited detection capabilities of instrumental techniques [13].

Solvent extraction or liquid-liquid distribution can achieve preliminary separation of tall oil components based on solubility differences. The solute distributes between two immiscible liquid phases due to variations in the strength of the interaction of solute and solvent molecules [13]. The lipophilic substances present in tall oil products have a stronger affinity towards nonpolar organic solvents.

Another preparative fractionation technique to isolate compounds based on solute-solvent interactions is thin-layer chromatography (TLC). Thin plates typically coated with silica gel are employed as the stationary phase, and compounds are separated based on the competition of the solute and the eluting solvent for binding places on the stationary phase [14]. TLC is fast, inexpensive, and requires simple instrumentation which makes it an excellent preparative technique for other sophisticated analytical techniques.

Solid-phase extraction (SPE) is a highly selective and versatile sample preparation method, which typically involves the use of commercially available packed polymer

or disposable glass cartridges to remove contaminants or fractionate the analytes prior to analysis [15]. In normal phase SPE, a polar stationary phase and a mid- to nonpolar matrix (e.g., acetone, n-hexane) are used to extract polar analyte(s) while in reversed phase SPE, a polar or moderately polar sample matrix and a nonpolar stationary phase are used to extract mid- to nonpolar analytes [16].

Different instrumental techniques have been suggested for the analysis of siloxanes. High-performance size exclusion chromatography (HPSEC), also called gel permeation chromatography (GPC), is a chromatographic method which is mainly employed for the distribution of molecules based on size and MWs. It is possible to identify and quantify low MW components present in silicone polymers [17]. HPSEC can be coupled with a low-temperature evaporative light-scattering detector (LT-ELSD) in which the eluent is nebulised into a fine aerosol, then the solvent is evaporated, and the compounds of interest are detected by measuring the amount diffracted light of the irradiation of the mist by a light source [18].

Gas chromatography (GC) is a method for the separation of organic substances based on volatility differences. A carrier gas allows substances to pass through packed or capillary columns. Less volatile substances are retained longer in the columns (longer retention times). When GC is coupled with a flame ionisation detector (GC-FID), precise quantification of volatile cyclic siloxanes is possible [17]. When coupled with a mass spectroscopy detector (GC-MS), identification of low MW species such as cyclic and linear siloxane oligomers indicate the presence of silicone polymers [17].

Inductively-coupled plasma mass spectrometry (ICP-MS) is a highly sensitive analytical technique that is capable of measuring trace metals in the parts per billion (ppb) and parts per trillion (ppt) concentration levels [19]. It is a suitable technique for measuring the total elemental Si concentration in tall oil products but the analysis suffers from severe C, N and O based polyatomic interferences on the masses of Si isotopes, that must be accounted for or eliminated.

In this work, analytical procedures were developed to preconcentrate silicone antifoam components in tall oil products and analyse organosiloxanes in the concentrated fraction via suitable instrumental techniques.

2. EXPERIMENTAL

2.1 Reagents and standard solutions

The following solvents and compounds were used in the preparation and analysis of silicone oil in tall oil products, as shown in Table 1.

Table 1. *Solvents and compounds used in the preparation and analysis of tall oil products.*

Solvent / Compound	Description	Manufacturer
methanol	HiPerSolv Chromanorm® gradient grade	VWR Chemicals (France)
n-hexane	HiPerSolv Chromanorm® HPLC	
acetone	AnalaR Normapur	
sulfuric acid (H_2SO_4)	AnalaR Normapur 95%	
phosphoric acid (H_3PO_4)	AnalaR Normapur ≥85%	
dichloromethane (DCM)	Puriss. p.a., ACS reagent, ≥99.9% (GC)	Merck KGaA (Germany)
ethyl acetate	puriss. p.a., ACS reagent, ≥99.5% (GC)	
methyl *tert*-butyl ether (MTBE)	EMSURE® ACS	
diethyl ether	EMSURE® ACS, ISO	
tetrahydrofuran (THF)	HPLC-grade inhibitor-free, ≥99.9%	
potassium hydroxide (KOH)	EMSURE®, pellets	
acetic acid	Analytical reagent grade, glacial	Fischer Scientific (UK)
toluene	HPLC gradient grade	
ethanol	ETAX min. 99.5% by weight	Altia Oyj (Finland)
Rhodamine B	98+% pure	ACROS Organics (Belgium)

Table 2 shows the different reagents used in the preparation of the internal standard and silylating solutions for GC-FID analysis. The detailed preparation of these solutions is described in the analytical procedures section (2.3.4).

Table 2. *Reagents used in the preparation of the internal standard and silylating solutions for GC-FID analysis.*

Reagent	Description	Manufacturer
betulinol	99.5% extract	-
cholesteryl heptadecanoate heneicosanoic acid 1,3-dipalmitoyl-2-oleoyl-glycerol	Sigma® Sigma® capillary GC approx. 99% Sigma® approx. 99%	Merck KGaA (Germany)
N,O – bis(trimethylsilyl) trifluoroacetamide (BSTFA)	98+%	ACROS Organics (Belgium)
chlorotrimethylsilane (TMCS)	≥98.0%	Merck KGaA (Germany)
pyridine	AnalaR Normapur	VWR Chemicals (France)

The organosiloxane sources were the following: an industrial laboratory formulation of an antifoam emulsion with 20% actives and a commercially available Aldrich silicone oil with a viscosity of 1000 centistokes (cSt) at 25ºC.

Table 3. *Solvents and compounds used in the preparation of sample, calibration, and internal standard solutions for ICP-MS analysis.*

Solvent / Compound	Description	Manufacturer
Si standard	1000 µg/mL in H_2O / 0.4% F^-, single-element	PerkinElmer (USA)
nitric acid (HNO_3) hydrogen peroxide (H_2O_2) hydrofluoric acid (HF)	Suprapur® 65% Suprapur® 30% Suprapur® 40%	Merck KGaA (Germany)
Rhodium (Rh) internal standard	Spectrascan® (1000 ± 2) ppm, 4.9 % HCl	Teknolab A/S (Norway)
distilled deionised water	18 MΩ·cm	ELGA

For the ICP-MS sample, calibration, and internal standard solutions preparation, the solvents and compounds shown in Table 3 were used.

2.2 Materials and instruments

Representative samples from four tall oil pitch (TOP 1 to 4), one crude tall oil (CTO 1), and four process samples (P1 to P4) from three industrial Finnish companies were placed in suitable containers. Kimax® 16 x 100 mm borosilicate glass culture tubes with threaded end were used in testing the different samples. HPLC/GC autosampler glass vials with 9 mm size, 2 mL capacity, and screw threads and caps served as sample vials for HPSEC and GC analysis.

VWR International 25 mm syringe filters with 0.2 μm polytetrafluoroethylene (PTFE) membrane were used to remove small particles. A 1000 μL borosilicate microsyringe was employed to transfer contents into autosampler vials. Eppendorf Multipipette® plus with 1-25 mL combitips were utilised in transferring organic solvents. 150 mm disposable glass Pasteur pipettes from VWR Chemicals (France) were used to separate the immiscible phases in test tubes.

TLC silica gel 60 F_{254} aluminium sheets (5 x 10 cm) were taken from Merck KGaA (Germany). Hirschmann® ringcaps® 5/10 μL capillary tips were purchased from Hirschmann Laborgeräte (Germany). 100 mg Strata® Si-1 silica cartridges with 60 μm average particle size and 61 Å pore size, and 1000 mg Thermo Scientific Hypersep SI cartridges with 40-63 μm irregular particles and 60 Å pore size were utilised for SPE analysis. An SPE manifold was used to fasten the silica cartridges, to maintain vacuum pressure, and to collect the desired sample fractions.

A Heraeus vacuum desiccator set at 40ºC was employed to remove moisture and volatile components. VWR International vortex mixer was used to mix the contents in test tubes properly. Heating of samples was done using a Memmert oven, and the centrifugation of test tubes was performed using a Sorvall® TC centrifuge. A PIERCE Model 18780 Reacti-Vap™ evaporating unit was employed to remove solvents in the sample. Solvent extraction mass measurements were performed using a Mettler Toledo XP205 analytical balance. Compressed air was used to remove volatile solvents adhering to test tubes and to Multipipette® combitips. Gas tanks of nitrogen (N_2), hydrogen (H_2), helium (He), and argon (Ar) were utilised for the evaporating unit, GC-FID, GC-MS, and ICP-MS analyses, respectively.

A Shimadzu LC-10AT liquid chromatograph combined with a Sedex 85 LT-ELSD was used for HPSEC analyses. The columns employed were Jordi Gel DVB 500 and Jordi X-stream H_2O mixed bed, both with an internal diameter of 7.8 mm and a length of 300 mm.

Clarus® 500 Gas Chromatograph and AutoSystem XL™ Gas Chromatograph from PerkinElmer (USA) were employed for short-column and long-column GC-FID, respectively. The columns used were the HP-1 Agilent Narrowbore GC column (short column) and the Agilent J&W Megabore GC column (long column). The HP-1 Agilent GC column has dimensions of 25 m length, 0.2 mm internal diameter, and a film thickness of 0.11 µm. The Agilent J&W GC column has a length of 6 m, 0.53 mm internal diameter, and a film thickness of 0.15 µm. HP 6890 Series GC system coupled with HP 5973 MS detector and an Agilent 19091Z-002 HP-1 methyl siloxane capillary column were used for GC-MS analyses.

For ICP-MS analyses, samples were weighed by using a Mettler AT261 DeltaRange® analytical balance. Polymethylpentene volumetric flasks, polypropylene sampling bottles, and Sarstedt tubes were used to prevent Si leaching when using borosilicate glassware. Thermo Scientific micropipettes were utilised to deliver oxidising acids and distilled water. Polytetrafluoroethylene (Teflon) microwave pressure vessels HF100 with lip-type seal screw caps were used during microwave-assisted acid digestion in Anton Paar Multiwave 3000 Microwave Sample Preparation System. All samples were analyzed by Perkin Elmer SCIEX ELAN DRC^{PLUS} ICP-MS with autosampler.

2.3 Analytical procedures

2.3.1 Solvent extraction

Initial phase separation of tall oil product samples was performed via solvent extraction using two immiscible solvents, methanol and n-hexane. Two sets of borosilicate culture tubes, labelled A and B, were prepared. Both test tube sets were

rinsed with ~3 mL of acetone. Acetone was removed by passing compressed air. The set A test tubes were placed in the oven at 70ºC for 2 minutes while the set B test tubes were placed in the vacuum desiccator at 40ºC for 20 minutes. Before placing the test tubes into the desiccator, their caps were slightly loosened.

250 mg of sample was weighed into set A test tubes. Set B test tubes were also weighed after being placed in a vacuum desiccator, which were used later to determine the mass of the extracted sample. 4 mL methanol, 4 mL n-hexane, and 100 µL of distilled water were added to all set A test tubes. Addition of distilled water improved the phase separation of the aqueous and organic fractions. The test tubes were thoroughly shaken using the vortex mixer. The test tubes were then centrifuged at a speed of less than 1500 rpm for five minutes.

The hexane-soluble phase (top fraction) was extracted using Pasteur pipettes to set B test tubes. A second 4 mL n-hexane was added to set A test tubes to improve extraction efficiency as batch solvent extraction is susceptible to adhesion of organic solvent to the test tube walls, requiring repeated washings [13]. The test tubes were again thoroughly shaken using the vortex mixer and then centrifuged for five minutes. The second hexane-soluble fraction was extracted and added to the first fraction.

The n-hexane solvent in set B test tubes was evaporated using the Reacti-Vap™ evaporating unit under N_2 gas at ~50ºC. The test tubes were placed in a vacuum desiccator for an hour at 40ºC. The tubes were then weighed, placed back into the vacuum desiccator at 30-minute intervals, and were weighed until a constant mass is obtained (<1% mass difference between two successive measurements). Sample weights were noted, and the mass of the extracted hexane-soluble fraction was compared against the initial sample mass.

2.3.2 Hexane-soluble fraction preparation

Five set B test tubes from the same sample were transferred to a 50 mL volumetric flask to prepare a stock solution of the hexane-soluble fraction. Each test tube was

adequately rinsed with n-hexane to transfer all contents into the flask. The solution was diluted to mark with n-hexane and was then transferred into two, clean 30 mL sample vials, which were used in further analyses.

The concentration of prepared stock solutions was determined gravimetrically by taking 1 mL of sample into pre-weighed, clean test tubes. The solvent was evaporated under N_2 gas at 50ºC. The total mass of the sample plus the test tube was compared against the mass of the test tube to determine its concentration.

2.3.3 High-performance size-exclusion chromatography (HPSEC)

From the hexane-soluble fraction stock solutions, 0.1 to 0.5 mL were transferred into clean, pre-weighed test tubes to obtain approximately 2 to 10 mg of sample. An equivalent volume of THF was added to all test tubes (e.g., 2 mg of the sample to 2 mL THF) to prepare 1 mg/mL sample solutions. The solutions were thoroughly mixed using a vortex mixer.

1000 μL of each sample was transferred to autosampler glass vials with plastic caps using a 500 μL microsyringe. While transferring the contents into the vials, the samples were filtered using a 0.2 μm PTFE filter to remove undesired particles. A blank sample with only 1000 μL of THF was also prepared to determine baseline and noise levels. The microsyringe was rinsed several times with THF to remove the contents of the previous sample.

The vials were loaded to the Shimadzu LC-10AT liquid chromatograph with HPLC-grade THF (1% v/v glacial acetic acid) as eluent. The eluent was prepared by adding 5 mL of filtered glacial acetic acid to 500 mL of THF. The flow rate was set to 0.8 mL/min, which corresponds to ~5.5 MPa column pressure, and the injection volume was 50 μL. The selected method was size-exclusion chromatography (28 minutes). The samples were analysed at 40ºC, sensitivity setting of Gain 3 or 6, and at constant mobile phase composition (isocratic flow).

The Jordi X-stream H_2O mixed bed column was used for the comparison of the antifoam emulsion and the 1000 cSt Aldrich silicone oil, while the Jordi Gel DVB 500 was used for all other HPSEC analyses. Chromatograms were generated by correlating the MWs of compounds present in the sample to the MW distribution of polystyrene.

A sensitivity test was performed by preparing different concentrations of Aldrich silicone oil solutions in THF (1.0 mg/mL to 0.0001 mg/mL with dilution factor: 1/3). The solutions were analysed using the Jordi Gel DVB 500 column at sensitivity values of Gain 3 and 6.

2.3.4 Gas chromatography-flame ionization detector (GC-FID)

1 mL of the HPSEC samples dissolved in THF was transferred to a separate, clean test tube. 2 mL of the 0.02 mg/mL internal standard solution (betulinol for sterols, TG standard for triglycerides, C_{21} fatty acid for free fatty acids and resin acids, and CH17 for steryl esters) was added to all samples.

The internal standard was prepared by taking 100 mg of each reagent, 99.5% betulinol extract, cholesteryl heptadecanoate, heneicosanoic acid, and 1,2-dipalmitoyl-2-oleoyl-glycerol, to obtain 1 mg/mL stock solutions in MTBE. 5 mL of each stock solution were combined in a 250 mL volumetric flask and diluted with MTBE resulting in a 0.02 mg/mL solution.

All test tube samples were placed in an evaporating unit under N_2 gas at 50ºC for ~20 minutes to evaporate THF and MTBE. The samples were then placed in a vacuum desiccator for 15 to 20 minutes. 150 µL of the silylation reagents, which consisted of 1 part pyridine, 4 parts BSTFA, and 1 part TMCS, were added to silylate the samples. Silylation is a derivatisation technique which converts the polar groups into nonpolar groups by replacing acidic hydrogen with an alkylsilyl group (e.g., $-SiMe_3$) enhancing the volatility and thermal stability of the nonpolar derivatives. Note that BSTFA and TMCS are moisture-sensitive and must be stored

in the refrigerator. Both reagents were allowed to cool down to room temperature first before opening.

All samples were placed in the oven at 70ºC for 5 minutes. The samples were shortly taken out and were mixed using a vortex mixer. The samples were then placed back in the oven for 40 minutes. After 45 minutes of heating, the samples were taken out and were allowed to cool down to room temperature and were remixed. The samples were transferred to pre-washed vials with V-shaped glass insert and plastic caps using Pasteur pipettes.

Clarus® 500 and AutoSystem XL™ gas chromatographs were employed to analyse the samples. Two rinse solutions were used: ethanol and toluene. The autosampler takes ethanol after analysing a sample while toluene is taken before the analysis of the next sample. The total run time per sample was 25.50 minutes.

2.3.5 Gas chromatography-mass spectrometry (GC-MS)

After GC-FID analysis, sample vials were loaded to the HP 6890 Series GC System with HP 5973 MS detector to obtain mass spectra of the samples. The instrument specifications used in the GC-MS analysis are shown in Appendix C Figure C1.

2.3.6 Alkaline hydrolysis

To improve the peak separation of high MW PDMS and steryl ester by HPSEC analysis, the TOP 4 hexane-soluble fraction was treated under alkaline conditions. Steryl esters hydrolyse under alkaline conditions to form free sterols which elute much later than steryl esters in HPSEC.

A 0.2 mL of TOP 4 hexane-soluble fraction stock solution was transferred into a clean test tube. The solvent was evaporated under N_2 gas at 50ºC. 2 mL of 0.5 M potassium hydroxide (KOH) in 90% ethanol was added to the test tube, and the contents were mixed. The sample was placed in an oven at 70ºC for 3 hours.

After heating, the sample was allowed to cool down to room temperature. 3 mL of distilled water and 4 mL of n-hexane were added. The test tubes were remixed and centrifuged for 5 minutes. The test tubes were then placed in a vacuum desiccator for ~20 minutes. Similar procedures to the solvent extraction (double extraction) were carried out to separate the hexane-soluble phase using Pasteur pipettes.

A 30% H_3PO_4 solution was prepared in a 10 mL beaker. 12-13 drops of the prepared H_3PO_4 solution was added to the alkali phase of the hydrolysed sample (pH ~3). The sample solution was mixed, and 4 mL of MTBE was added. The test tubes were centrifuged for 5 minutes, and the MTBE-soluble phase was extracted. 4 mL of MTBE was again added, and the extraction for the MTBE-soluble phase is repeated (double extraction).

The extracted samples, i.e., the hexane-soluble and MTBE-soluble phases, were dissolved in THF and were analysed by HPSEC and GC-FID to determine the effect of alkali addition to TOP samples.

2.3.7 Thin-layer chromatography (TLC)

Preliminary solvent optimisation tests were carried out via TLC to determine the most suitable solvent combination with the most enhanced separation of the different TOP components. Since TLC is a fast technique, several sets of samples can be tested simultaneously with minimal solvent volume requirement.

Different solvent combinations using n-hexane, diethyl ether, and MTBE were prepared in a 250 mL beaker. The silica plates were initially marked with a straight line by a pencil wherein 5 μL of sample was applied using capillary tips. The samples tested were a 50 mg/mL Aldrich silicone oil solution in hexane, the unhydrolysed and hydrolysed TOP 4 sample fractions, and reference standard solutions of steryl esters and sitosterol.

The plates were placed in contact with the prepared solvent. The solvent was allowed to elute until no significant change in solvent height is noticed. The highest point reached by the movement of the solvent and the sample was marked to determine the retention factors. The plates were removed from the beaker and were dried to remove remaining solvent.

The plates were initially visualised under ultraviolet light at 254 nm, and the eluted components were marked. The plates were then sprayed with 25% v/v aqueous sulfuric acid in ethanol solution or rhodamine B in ethanol solution. The plates were heated at 150 ºC for 1-3 minutes to char and localise the spots. The charred sample components were marked, and retention factors were calculated.

2.3.8 Solid-phase extraction (SPE)

The hexane-soluble TOP sample fraction was further fractionated by SPE to extract the high MW PDMS, preferably in one fraction. Various solvent concentration combinations of the most suitable solvents based on TLC results were prepared in 100 mL beakers. Hypersep SI cartridges were placed onto the extraction manifold, and test tubes were placed for collecting the eluates. The vacuum line was connected, and the vacuum knob was switched on to control the vacuum pressure within the system.

The cartridge was conditioned first with DCM and then n-hexane as wash solvent. 1.5 mL of the TOP 4 hexane-soluble fraction was loaded. Afterwards, the first elution solvent was loaded, and the eluate was collected for analysis. The loading of the elution solvent and collection of eluates were continued until the last elution solvent. All test tubes were collected, and the solvents were evaporated under N_2 gas at 50ºC. The collected eluates were dissolved in THF and were analysed by HPSEC and GC-FID to determine the components of each fraction.

2.3.9 Inductively-coupled plasma mass spectrometry (ICP-MS)

ICP-MS analyses were carried out to TOP samples and their corresponding hexane- and methanol-soluble fractions to determine the total elemental Si concentration of each sample and the Si distribution after solvent extraction.

200 to 250 mg of TOP samples were weighed into clean, labelled Teflon microwave vessels. 5 mL of oxidising acid HNO_3 and 1 mL of H_2O_2 were then added to each vessel to dissolve the sample. In some experimental runs, 10 µL of HF was added to test its effect on the Si concentration. Blank samples were also prepared, with only acids added to the vessel, to estimate the baseline Si concentrations.

The vessels were transferred to the rotor body, and the rotor was placed into the microwave chamber. A decomposition program for simple organic samples (Farmabetain) was selected. The method selection was based on the total number of samples, as shown in Table 4.

Table 4. *Farmabetain digestion methods specifications*

Method	Power, W	Ramp time, min	Hold time, min	Number of samples
HF 100-8	800	15	20	4-8
HF 100-10	1000	15	20	8-10
HF 100-16	1400	15	20	10-16

The microwave digestion was set to run for approximately 60 minutes. The temperature of each reaction vessel was monitored by an external infrared sensor.

After digestion, the contents of each microwave vessel were transferred to a 100 mL volumetric flask and diluted to the mark with distilled water. The contents were transferred to sampling bottles, and further dilution was performed by taking 2 mL of sample and 8 mL of distilled water into Sarstedt tubes (Dilution factor: 1/5).

Si standard calibration solutions in 1 % v/v HNO_3 acid were prepared from the 1000 µg/mL single-element stock Si standard. The detailed preparation of calibration solutions in 1 % v/v HNO_3 acid is described in Table 5.

Table 5. *Silicon standard calibration solutions preparation (10 ppb to 10 ppm)*

Si Standard concentration	Description[a]
10 ppm	1 mL of 1000 µg/mL stock Si standard
1 ppm	10 mL of 10 ppm Si standard
200 ppb	2000 µL of 10 ppm Si standard
100 ppb	1000 µL of 10 ppm Si standard
50 ppb	500 µL of 10 ppm Si standard
25 ppb	250 µL of 10 ppm Si standard
10 ppb	1000 µL of 1 ppm Si standard

[a]All Si standard solutions are prepared in 100 mL volumetric flasks and are added with 1 mL HNO_3 acid and diluted to mark with distilled deionised water.

All standard solutions were transferred to sampling bottles, and approximately 10 mL was taken into Sarstedt tubes for analysis.

A 20 ppb Rh standard was prepared from the stock (1000 ± 2) ppm Rh internal standard with 4.9 % HCl. The 20 ppb internal standard solution was transferred to its container with a line connection to the ICP-MS sample introduction system.

The cooling water was allowed to flow before turning on the plasma to maintain the temperature of ICP-MS components during analysis. The samples and standard calibration solutions were placed into the autosampler. The peristaltic pump lines were fastened and the optimised pump settings, specified in Table 6, was set to allow sufficient washing in between sample measurements.

Table 6. *Optimised peristaltic pump specifications during ICP-MS operation*

Sequence	Time, s	Speed, ± rpm
Sample Flush	35	-24.0
Read Delay	15	-18.0
Analysis	-	-18.0
Wash	60	-24.0

The ICP-MS instrument specifications, as shown in Table 7, were presented in the Instrument window. The plasma was turned on and was allowed to stabilise before running blank solutions.

Table 7. *SCIEX ELAN DRC^{PLUS} ICP-MS specifications*

Parameter	Value	Unit
Vacuum Pressure	7.6×10^{-6}	torr
Nebulizer Gas Flow	0.88	L/min
ICP RF Power	1100	W
Lens Voltage	5.5	V
Analog Stage Voltage	-1600	V
Pulse Stage Voltage	800	V

Table 8 specifies the different parameters for the method timing (sweeps, readings per replicate, and the number of replicates). These parameters were optimised to obtain better statistics.

Table 8. *Optimised method timing specifications during ICP-MS operation*

Parameter	Number	Estimated time, sec
Sweeps	15	3.09
Reading/Replicate	1	3.09
Replicates	9	27.81

Si and Rh were added as analytes with Rh as the internal standard, and both elements were scanned in peak hopping mode. The selected method measured signal based on the processing specifications shown in Table 9.

Table 9. *Method processing specifications during ICP-MS operation*

Parameter	Description/Value
Detector	Dual
Process Spectral Peak	Average
AutoLens	On
Blank Subtraction	After Internal Standard
Process Signal Profile	Average
Measurement Unit	counts per second (cps)
Smoothing Factor	5

The analysis was started by continuously analysing blank solutions until the difference in both Si and Rh measured mean intensity was <500 cps. Then, the calibration standard solutions were analysed considering the desired correlation coefficient (R) value of >0.9999. The samples were analysed, and the results were reprocessed to obtain Si concentrations in µg/kg.

3. RESULTS AND DISCUSSION

3.1 Solvent extraction

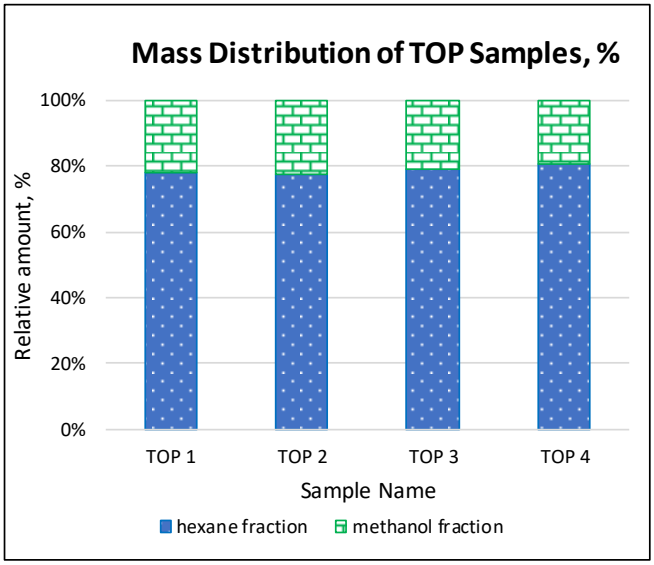


Figure 6. *Relative amounts of hexane- and methanol-soluble fractions of the four TOP samples post-solvent extraction expressed as weight per cent.*

The hexane-soluble components of the four TOP samples were extracted via solvent extraction using n-hexane and methanol. The properties of n-hexane and methanol with water as a reference is shown in Appendix A Table A1. Figure 6 presents the average mass percentages of the extracted hexane- and methanol-soluble fractions of the four TOP samples determined by gravimetry. 77.7-80.5% of each TOP sample was extracted to the hexane phase while only 19.5-22.3% were collected in the methanol fraction. The detailed gravimetric result of the mass distribution is shown in Appendix A Table A2.

Table 10. *Average concentrations of the hexane-soluble TOP fraction stock solutions*

Sample Name	Concentration[a], mg/mL
TOP 1	19.89
TOP 2	19.75
TOP 3	19.72
TOP 4	20.29

[a]determined by gravimetry

The concentration of the prepared stock solutions of hexane-soluble TOP fractions was determined gravimetrically and is summarised in Table 10 (see Appendix A Table A3 for the calculations). The average concentration is approximately 20 mg/mL for all TOP samples.

3.2 HPSEC

Initial HPSEC analysis of the four TOP samples was carried out to determine their composition. Chromatograms, as shown in Figure 7, reveal the presence of steryl esters, fatty acids, sterols, and resin acids in the TOP samples dissolved in THF. Steryl esters were the main components eluted at residence time (RT): 18-21.5 minutes. The four TOP samples slightly differ in their contents, with TOP 1 and TOP 2 containing more fatty acids, sterols, and resin acids as compared to TOP 3 and TOP 4.

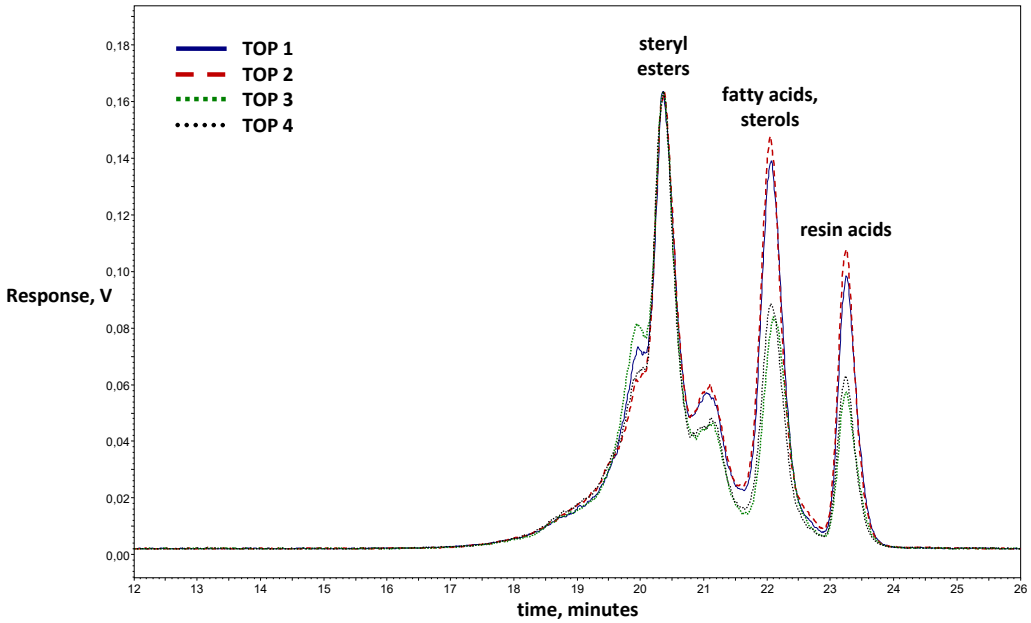


Figure 7. *HPSEC chromatograms of the four TOP samples showing the elution of steryl esters, fatty acids, sterols, and resin acids (Gain 3).*

Figures 8 and 9 show the HPSEC analyses of the hexane-soluble and the methanol-soluble TOP components post-solvent extraction. The chromatograms

depict that almost all steryl esters were present in the hexane-soluble fraction while the fatty acids, sterols, and resin acids were distributed between the two fractions.

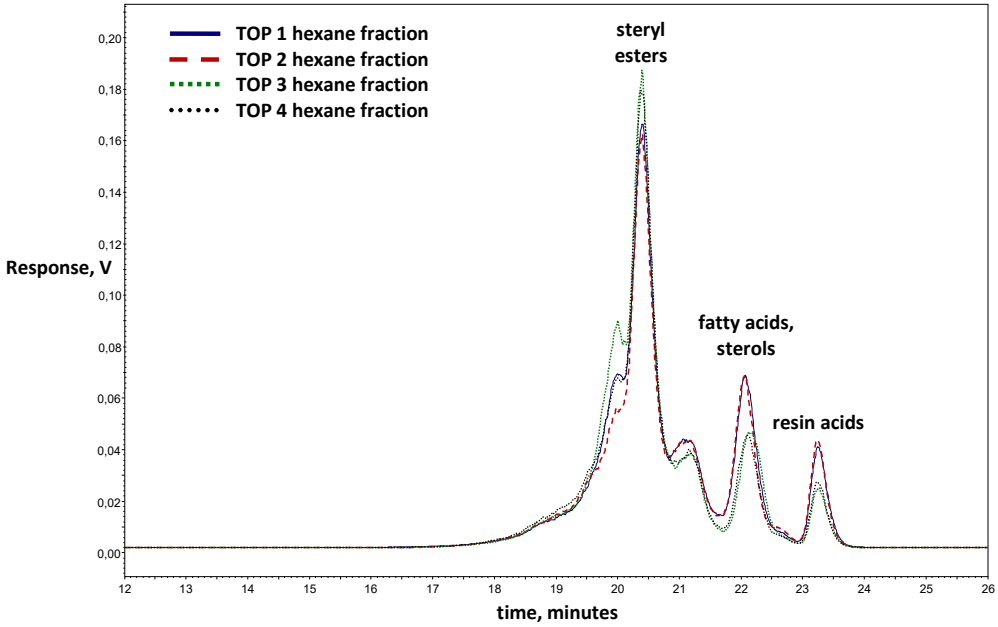


Figure 8. *HPSEC chromatograms of the four hexane-soluble TOP sample fractions showing the elution of steryl esters, fatty acids, sterols, and resin acids (Gain 3).*

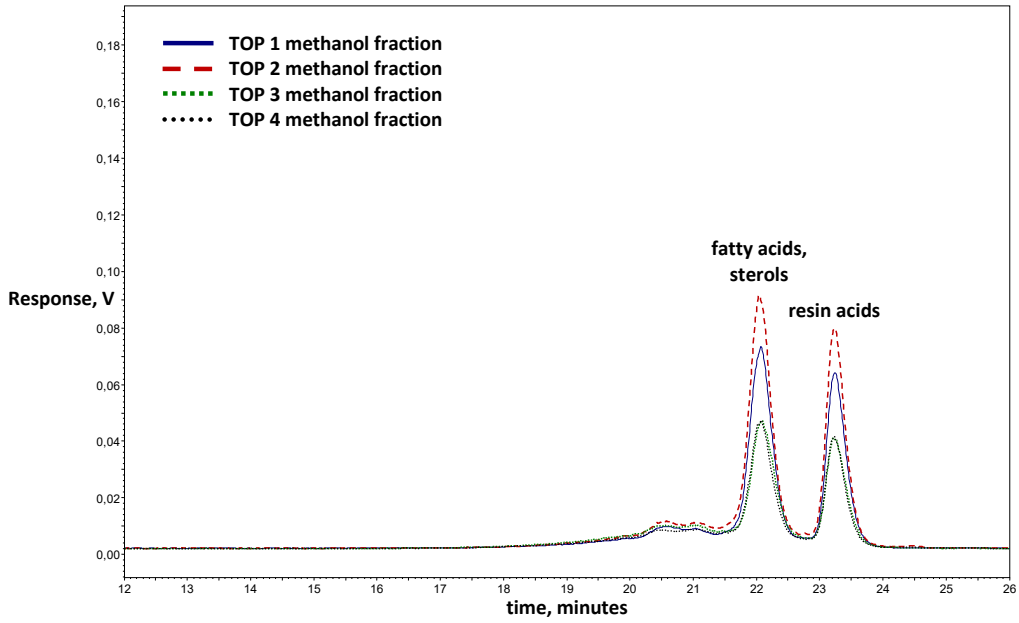


Figure 9. *HPSEC chromatograms of the four methanol-soluble TOP sample fractions showing the elution of fatty acids, sterols, and resin acids (Gain 3).*

A direct comparison between a TOP sample, TOP 4, and its corresponding methanol- and hexane-soluble fractions is shown in Figure 10. These chromatograms confirm the preliminary separation of some sterols, fatty and resin acids from steryl esters by solvent extraction.

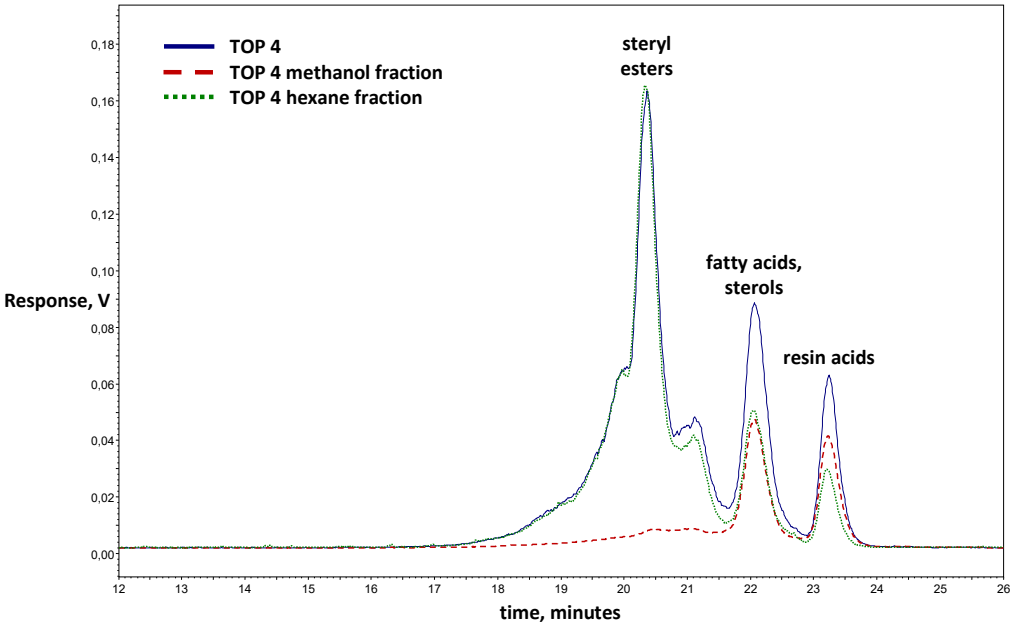


Figure 10. *HPSEC chromatograms of the TOP 4 sample and its hexane- and methanol-soluble fractions (Gain 3).*

Holmbom and Erä [7] investigated six grades of TOP, four of Finnish origin and two of US origin. The investigated samples contained 34.6-51.6% free acids (dehydroabietic, abietic, and other resin acids), 23.2-37.8% esterified acids (oleic and linoleic acids), and 25.3-34.4% unsaponifiable neutral compounds (diterpene alcohols, fatty alcohols, sterols, and dehydrated sterols). For samples TOP 1-4, the high unsaponifiables content likely indicates the use of hardwood (e.g., birch) in pulping.

Two standard solutions of the commercially available Aldrich silicone oil in n-hexane were prepared, with concentrations of 0.1 mg/mL and 1.0 mg/mL. Figure 11 illustrates the comparison among all the hexane-soluble TOP sample fractions and the Aldrich silicone oil solutions. The high MW PDMS were detected in both Aldrich silicone oil solutions at RT: 13-17 minutes.

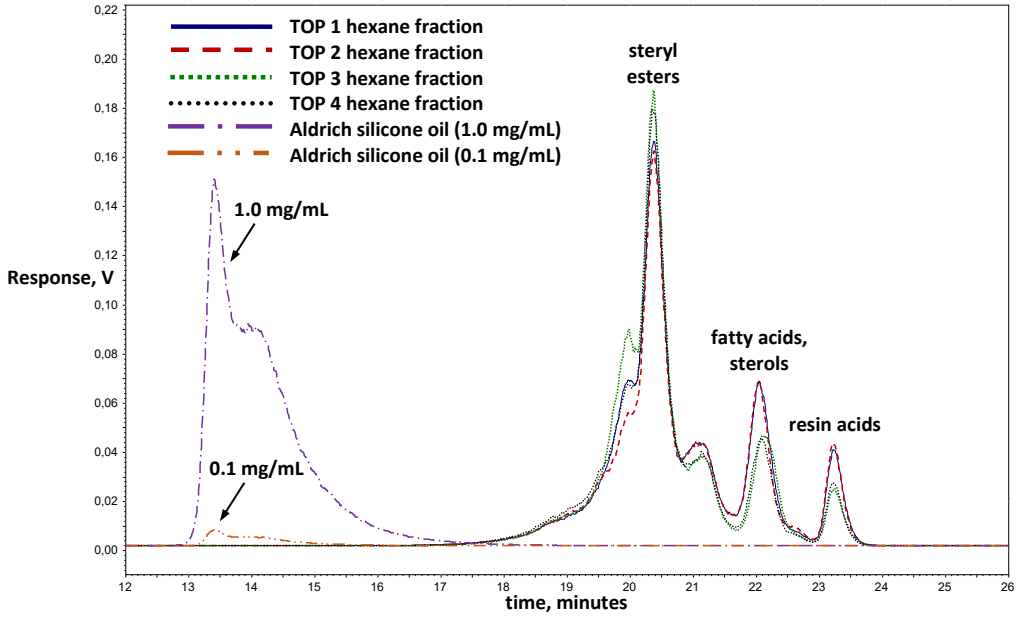


Figure 11. *HPSEC chromatograms of the four hexane-soluble TOP sample fractions and two standard solutions of Aldrich silicone oil in n-hexane (Gain 3).*

Analysis of the TOP 4 hexane-soluble fraction spiked with 50 mg of Aldrich silicone oil solution in n-hexane, presented in Figure 12, reveal that the high MW PDMS elute together with the hexane-soluble TOP components. The collection of the high PDMS in the hexane fraction indicates the removal of some polar compounds present in the TOP.

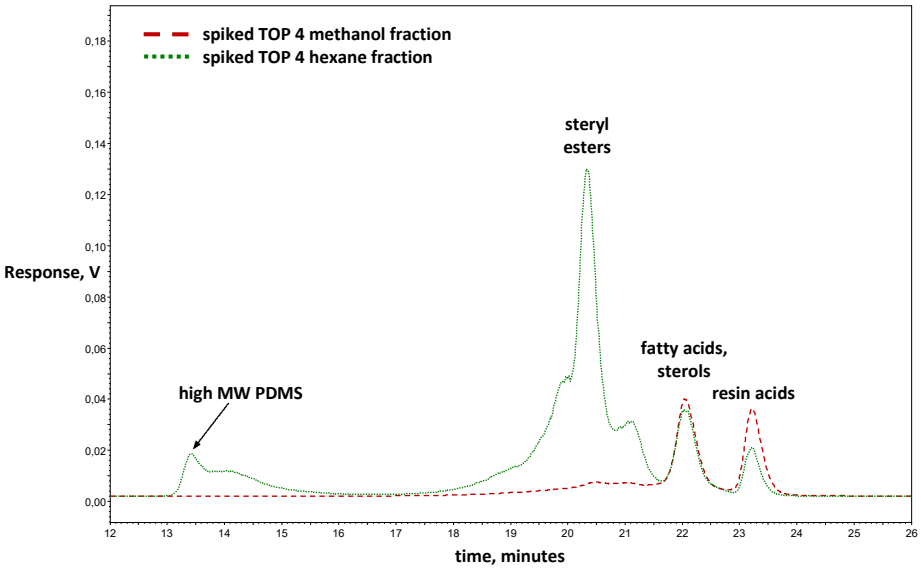


Figure 12. *HPSEC chromatograms of the hexane- and methanol-soluble fractions of the TOP 4 sample spiked with 50 mg Aldrich silicone oil (Gain 3).*

HPSEC analysis of the hexane-soluble fractions of CTO 1 and the four process samples (P1 to P4) was performed and is visualised in Figure 13. The P2 sample was predominantly fatty acids. The P1, P4, and CTO 1 samples were mainly fatty and resin acids while the P3 sample closely resembled the TOP samples.

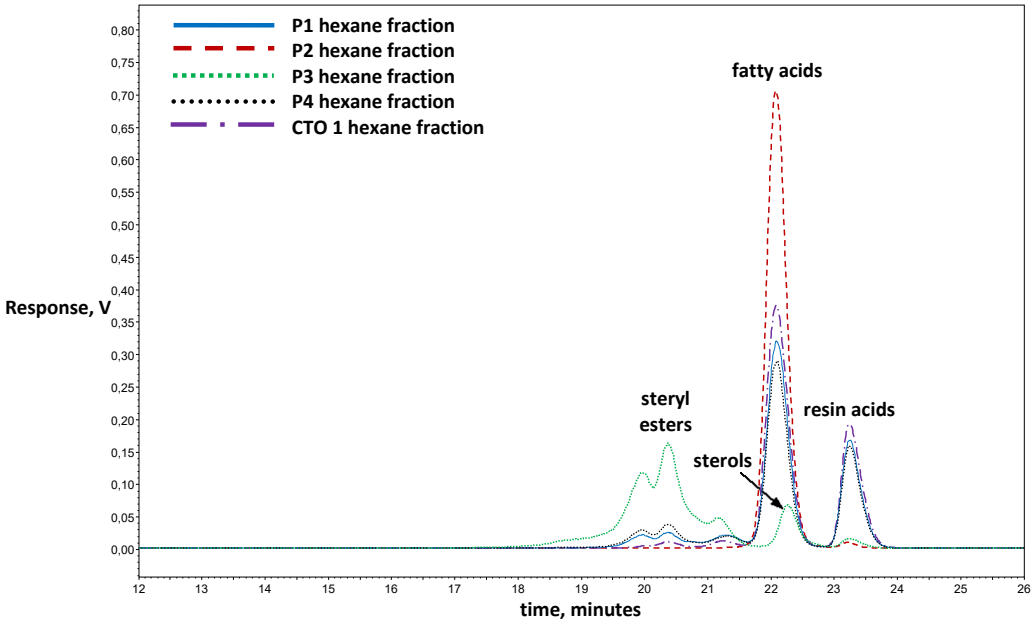


Figure 13. *HPSEC chromatograms of the hexane-soluble fractions of CTO 1 and the process samples P1 to P4 (Gain 3).*

The sensitivity test results of the different concentrations of Aldrich silicone oil in THF solutions at sensitivity values of Gain 3 and 6 are shown in Appendix B Figures B1.1, B1.2, B2.1, and B2.2. At Gain 3, the minimum concentration that can be detected for PDMS is at 0.012 mg/mL while at Gain 6, it is at a much lower concentration at 0.001 mg/mL, although the noise levels are higher at higher sensitivity.

Figure 14 shows a comparison between the antifoam emulsion with 20% actives and the Aldrich silicone oil solution by HPSEC. Both materials contain the high MW PDMS, RT: 13-17 minutes, but some low MW antifoam components elute at RT: 21-22 min for the antifoam emulsion. These low MW components will be evaluated in future experiments.

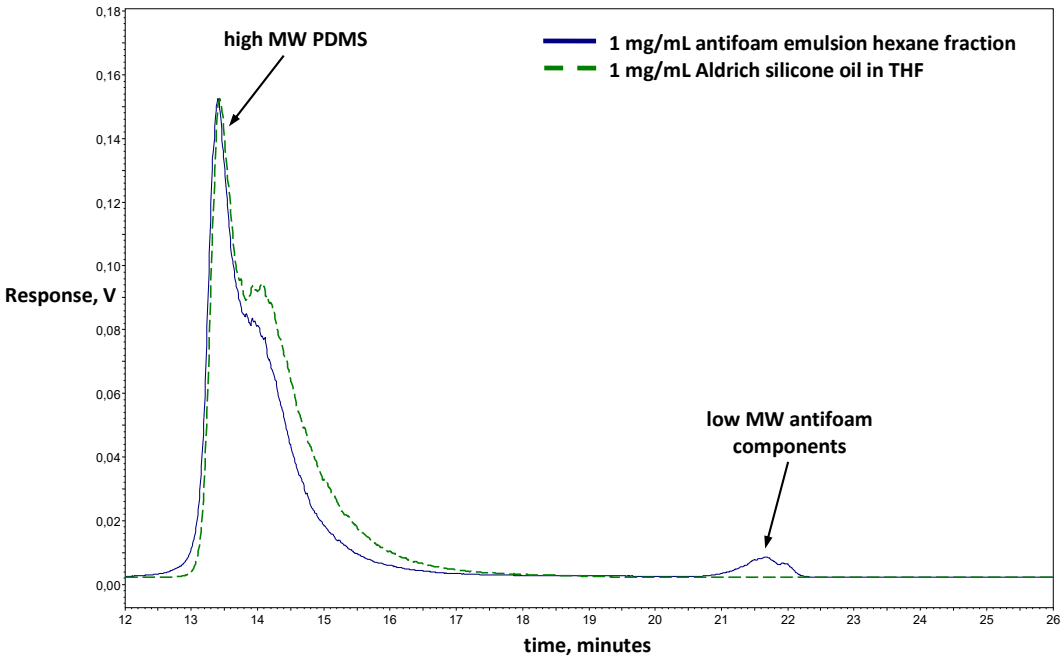


Figure 14. *HPSEC chromatograms of 1 mg/mL antifoam emulsion hexane-soluble fraction and 1 mg/mL Aldrich silicone oil solution in THF (Gain 3).*

To further compare the antifoam emulsion and the Aldrich silicone oil, molecular weight curves were generated using the Jordi X-stream H_2O mixed bed column. The differential MW curve, as seen in Figure 15, indicates that the antifoam emulsion and Aldrich silicone oil had similar MW curves. The integral molecular weight curve is shown in Appendix B Figure B3. These MW curves were generated based on the calibration curve of polystyrene (Appendix B Figure B4 and Table B1).

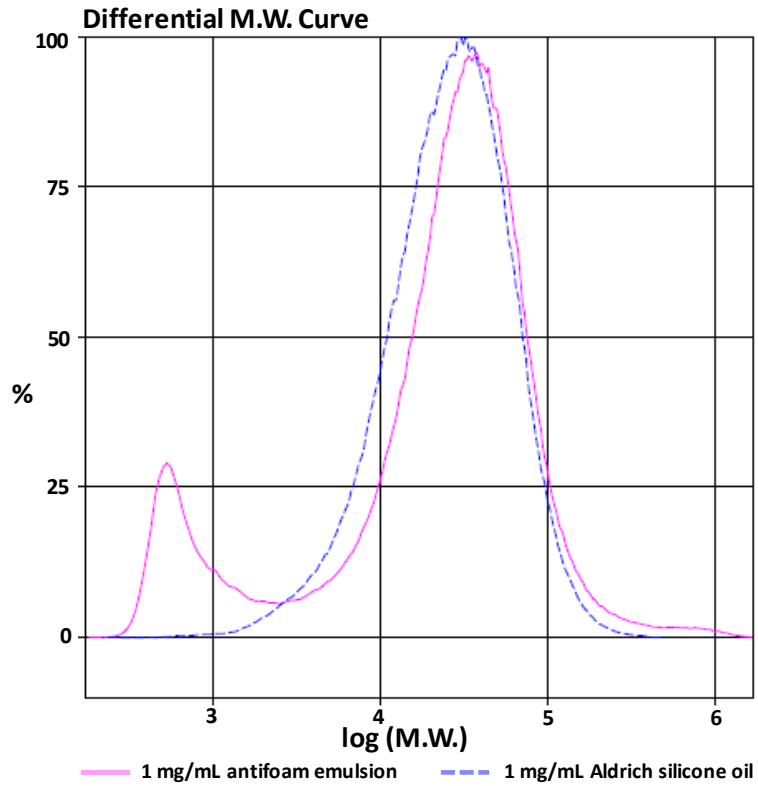


Figure 15. *Differential molecular weight curves of 1 mg/mL antifoam emulsion hexane-soluble fraction and 1 mg/mL Aldrich silicone oil solution in THF.*

Table 11 shows the numerical GPC results of both materials. The weight average MW of the antifoam emulsion is 48 kDa while the Aldrich silicone oil is 38 kDa. Both materials fall under the high MW PDMS classification (>30 kDa). It is worth noting that the low MW components which are present in the antifoam emulsion contributed to its high polydispersity value or M_w/M_n of 6.9.

Table 11. *The average molecular weight of the antifoam emulsion and the Aldrich silicone oil*

Sample	Time Shift	M_n	M_w	M_n/M_w
antifoam emulsion	0.0	7024	**48320**	6.9
Aldrich silicone oil	0.0	19228	**38009**	2.0

M_n - number average molecular weight
M_w - weight average molecular weight
M_n/M_w – polydispersity

3.3 GC-FID

A short column GC-FID analysis was carried out for all samples to confirm the components registered in the HPSEC analysis. GC-FID analysis of the hexane-soluble TOP fractions recorded similar chromatograms among all samples as seen in Figure 16. Slight differences in C_{18} fatty acid, RT: 5-5.8 min, and sitosterol, RT: 11 min, contents were observed.

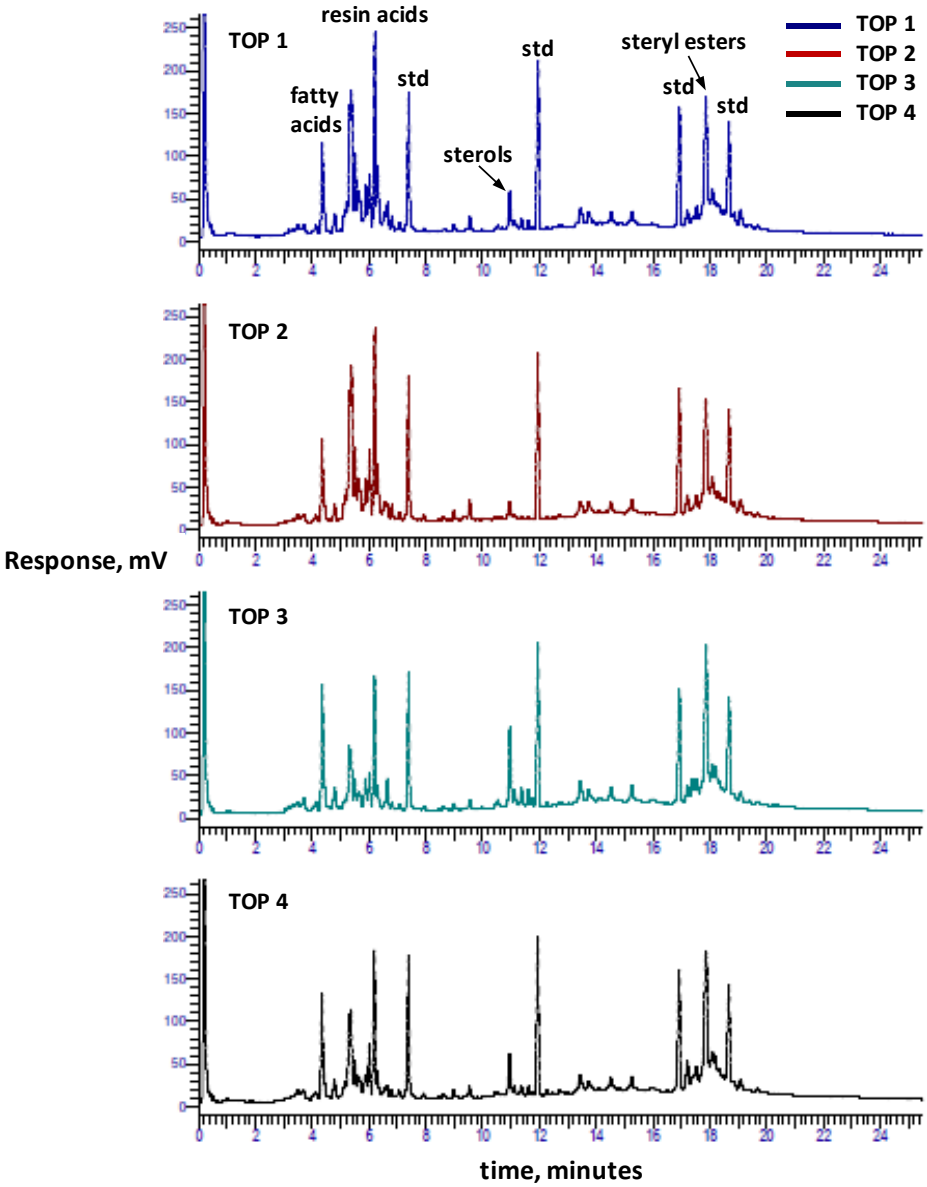


Figure 16. *Short-column GC-FID chromatograms of the four hexane-soluble TOP sample fractions showing the elution of TOP components and the compounds present in the internal standard solution.*

Short column GC-FID analysis of process samples P1, P2, and P4, as shown in Figure 17, confirms the presence of free fatty acids, resin acids, sterols, and small amounts of steryl esters in both P1 and P4 samples. The P2 sample contained mostly fatty acids with an intense peak at RT: ~5.5 minutes.

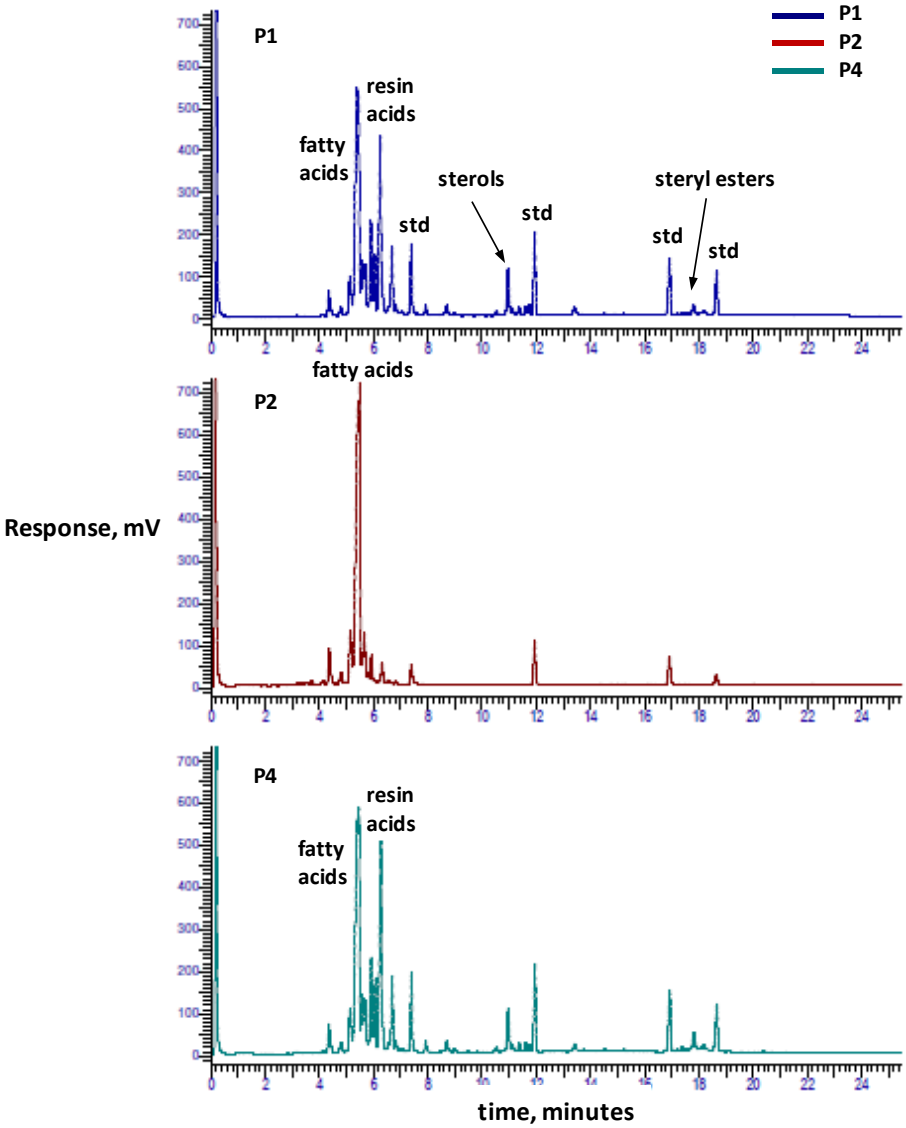


Figure 17. *Short-column GC-FID chromatograms of the hexane-soluble fractions of process samples P1, P2, and P4.*

Figure 18 shows the GC-FID analysis of the process sample P3, CTO 1, and the 1.0 mg/mL Aldrich silicone oil in n-hexane solution. The P3 sample contained steryl esters and sterols, similar to the TOP samples. The CTO 1 sample contained free fatty acids, resin acids, sterols and small amounts of steryl esters. For the 1.0

mg/mL of Aldrich silicone oil solution, no significant peak was registered as only standard solution peaks were detected.

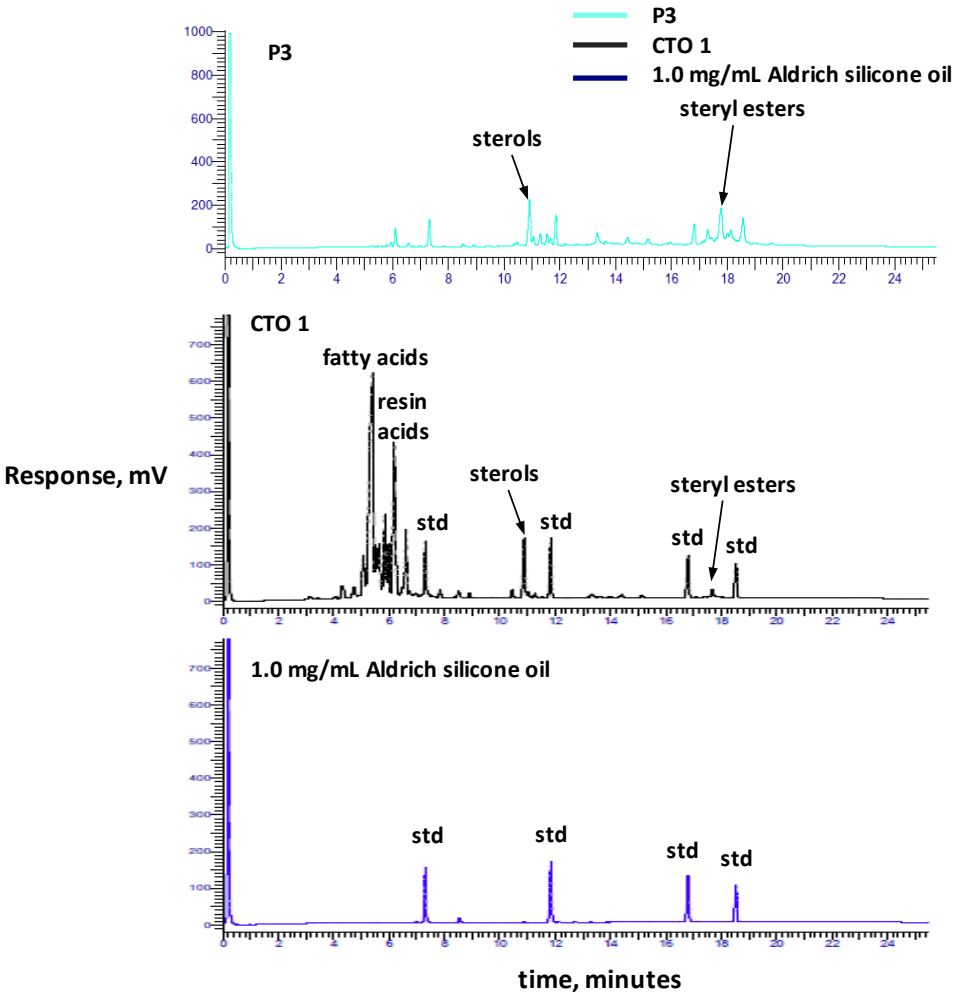


Figure 18. *Short-column GC-FID chromatograms of the hexane-soluble fractions of P3, CTO 1, and the 1.0 mg/mL Aldrich silicone oil solution in n-hexane.*

3.4 Alkaline hydrolysis

Alkaline hydrolysis of the TOP 4 hexane-soluble fraction using 0.5 M KOH resulted in the hydrolysis of the majority of steryl esters to free sterols, primarily sitosterol. The acidic components, fatty and resin acids, remained in the alkali-water phase. Fatty and resin acids are neutralised when reacted with an alkali solution (e.g., KOH) to form ionic salts and water [3,5]. Potassium and sodium salts are readily soluble in water.

Figure 19 shows the comparison of a hydrolysed hexane-soluble TOP 4 sample fraction against an unhydrolysed fraction. It is evident that the chromatogram is shifted to the right, i.e., longer retention times by HPSEC analysis. This shift is a desirable situation as the hydrolysed TOP components elute much later than the high MW PDMS components.

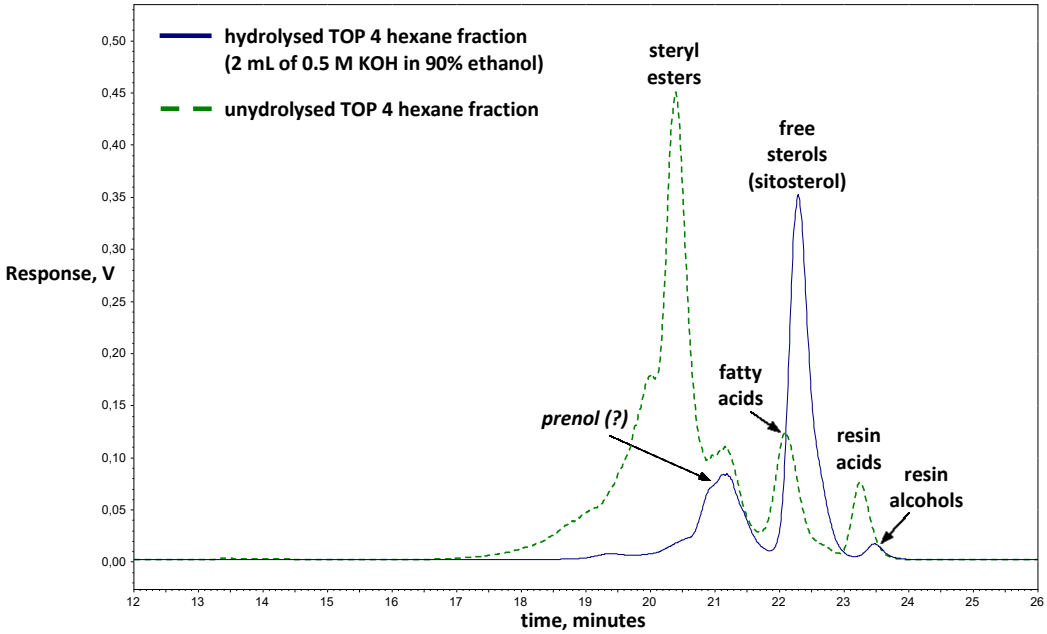


Figure 19. *Alkaline hydrolysis result of the reaction of 2 mg/mL TOP 4 hexane-soluble fraction and 2 mL of 0.5M KOH solution in 90% ethanol (Gain 3).*

A GC-FID comparison of the hydrolysed and unhydrolysed hexane-soluble TOP 4 fractions is shown in Figure 20. Steryl esters were hydrolysed to free sterols, RT: ~11 min. Resin alcohols (RT: 4 to 6 min), C_{22} alcohol (RT: ~7.5 min), and C_{24} alcohol (RT: ~8.5 min) are formed during alkali addition. Between RT: 3 to 4 minutes, some interesting non-polar, low MW, non-hydrolysable TOP components elute. It is recommended to investigate further these interesting components. The GC-MS result of the hydrolysed hexane-soluble TOP 4 fraction (Appendix C Figure C2) corroborated the results obtained from GC-FID.

The HPSEC and short column GC-FID chromatograms of the MTBE-soluble hydrolysed TOP 4 fraction is presented in Appendix D Figures D1 and D2, respectively.

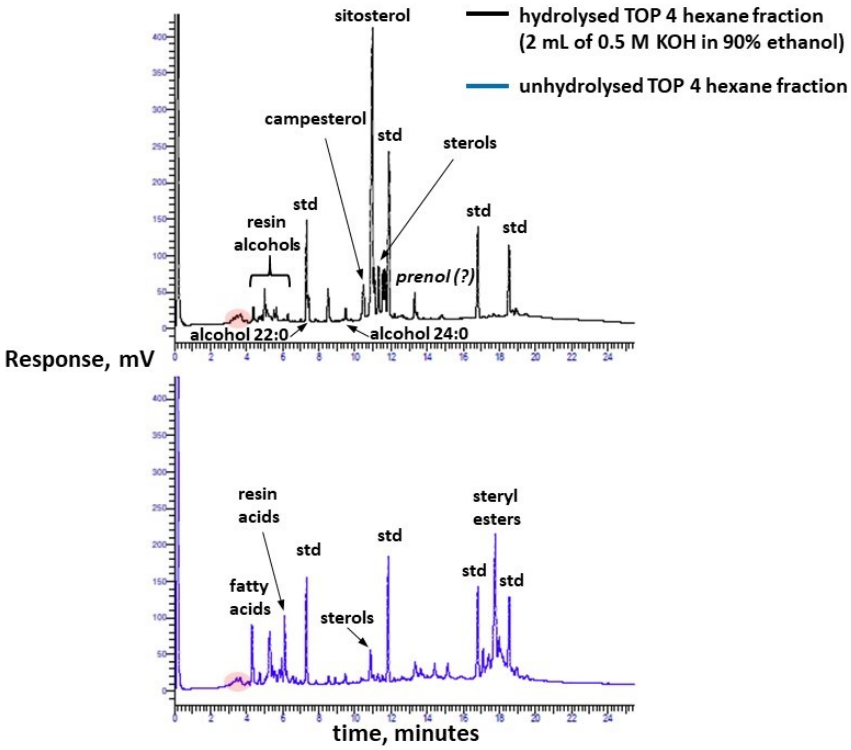


Figure 20. *Short-column GC-FID chromatograms of the hydrolysed and unhydrolysed TOP 4 hexane-soluble fractions.*

As the HPSEC chromatogram for the hexane-soluble TOP 4 fraction is shifted away from the retention time for the high MW PDMS, alkaline hydrolysis is a promising step in concentrating PDMS. However, upon hydrolysis of the Aldrich silicone oil in n-hexane solution, presented in Figure 21, the high MW PDMS components were also hydrolysed into different lower MW components. The sharp peak from 13 to 17 minutes disappeared, which indicate partial or complete hydrolysis of the Aldrich silicone oil. The degradation of the high MW PDMS to low MW siloxane is also confirmed by GC-MS analysis (Appendix C Figure C3).

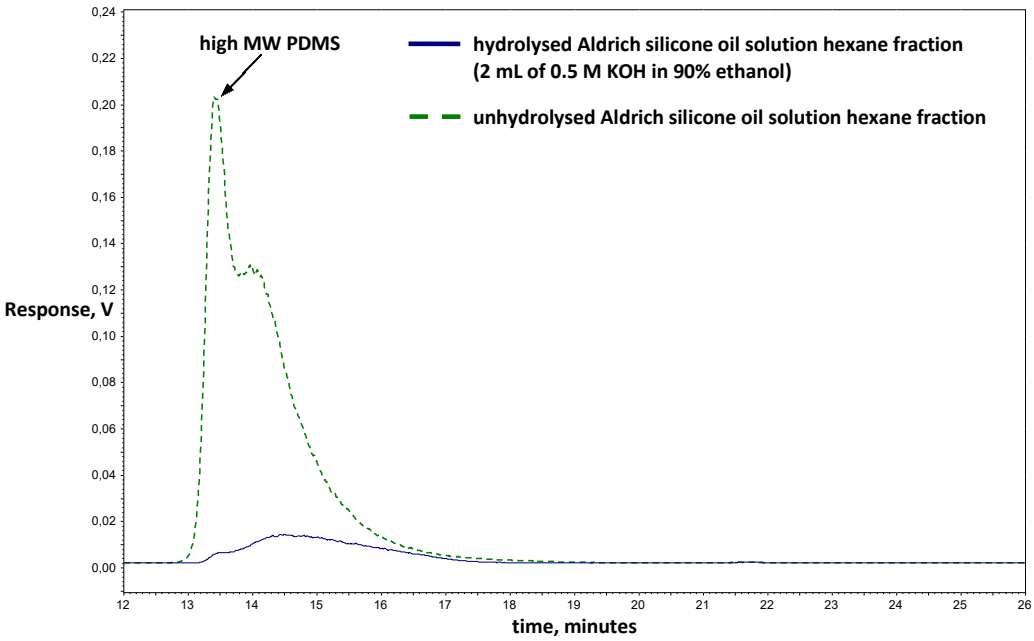


Figure 21. *Alkaline hydrolysis result of the reaction of 1 mg/mL Aldrich silicone oil hexane-soluble fraction and 2 mL of 0.5M KOH solution in 90% ethanol (Gain 3).*

A long column GC analysis of the MTBE-soluble fraction of the hydrolysed Aldrich silicone oil solution, presented in Figure 22, confirmed the hydrolysis of the high MW PDMS into low MW siloxanes or the formation of linear and cyclic oligomers as stated in Eq. 2.

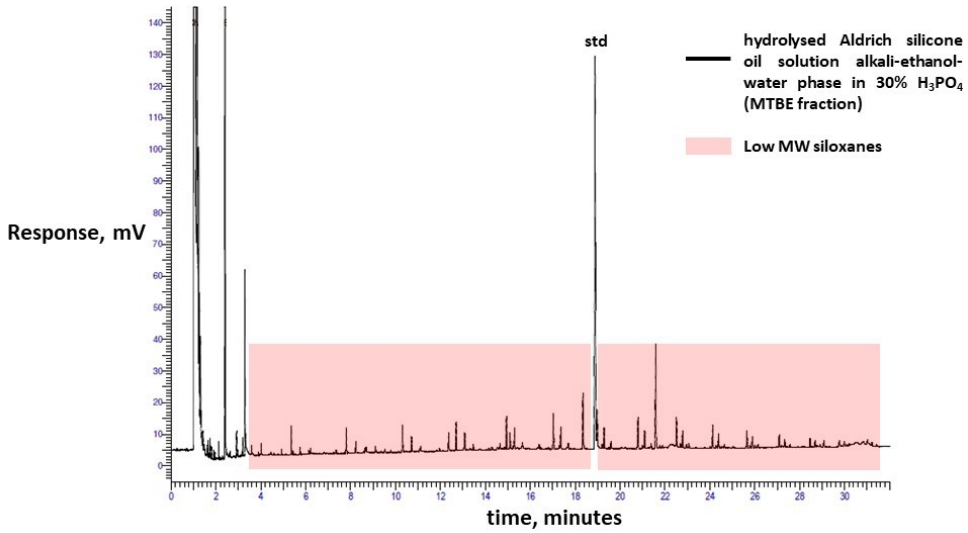
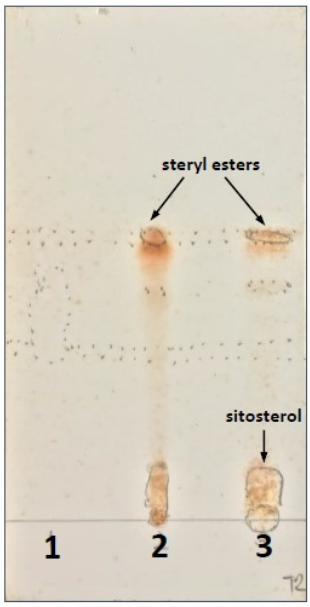


Figure 22. *Long-column GC-FID chromatogram of the MTBE-soluble fraction of 1 mg/mL hydrolysed Aldrich silicone oil solution upon addition of 30% H_3PO_4 acid.*

3.5 Thin-layer chromatography

Figure 23 depicts the TLC results of three samples: a 50 mg/mL Aldrich silicone oil in n-hexane solution, a TOP 4 hexane-soluble fraction, and a 32 mg/mL hydrolysed TOP 4 hexane-soluble fraction using an eluent with 85% v/v n-hexane and 15% v/v diethyl ether.



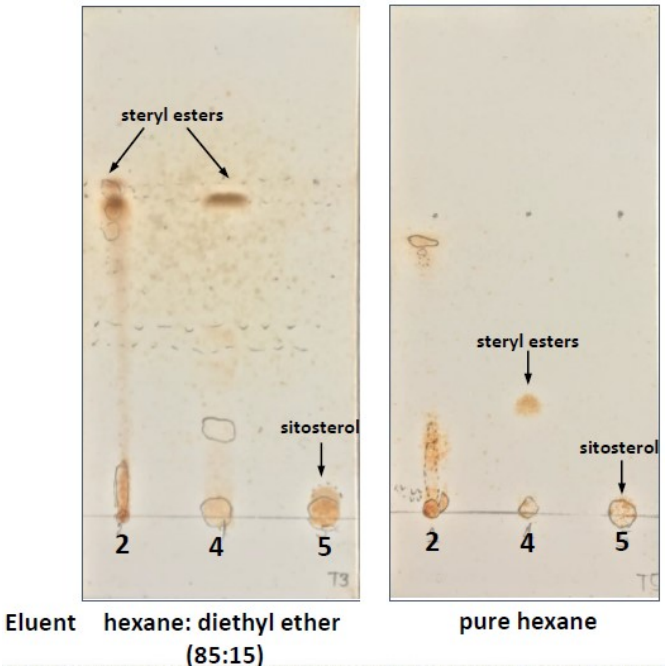
Eluent: hexane: diethyl ether (85:15)

1: 50 mg/mL Aldrich silicone oil in n-hexane solution
2: TOP 4 hexane-soluble fraction
3: 32 mg/mL hydrolysed TOP 4 hexane-soluble fraction

Figure 23. *TLC results of a 50 mg/mL Aldrich silicone oil solution in n-hexane (1), unhydrolysed (2), and hydrolysed (3) TOP 4 hexane-soluble fractions showing the conversion of steryl esters to sitosterol (sprayed with 25% v/v aqueous sulfuric acid in ethanol solution).*

As seen from the figure, the steryl esters in the TOP 4 hexane-soluble fraction (2) eluted fast with retention factor R_f ~1. Some of the steryl esters were hydrolysed to sitosterols in the hydrolysed TOP 4 hexane-soluble fraction (3). The components of the Aldrich silicone oil solution (1) were not visible even after spraying with sulfuric acid in ethanol solution. The results of using a fluorescent reagent, Rhodamine B, as spray solution is presented in Appendix E Figure E1.

Steryl ester and sitosterol standard solutions were prepared as the reference for TLC analysis. Figure 24 presents the TLC results of the unhydrolysed TOP 4 hexane-soluble fraction (2) and steryl esters (4) and sitosterol (5) reference standard solutions in two different solvent mixtures: 85% v/v n-hexane and 15% v/v diethyl ether, and pure hexane.



2: TOP 4 hexane-soluble fraction
4: steryl ester reference standard
5: sitosterol reference standard

Figure 24. *TLC results of the unhydrolysed TOP 4 hexane-soluble fraction (2) compared against a steryl ester (4) and sitosterol (5) reference standards (sprayed with 25% v/v aqueous sulfuric acid in ethanol solution).*

Results were comparable to Figure 23 for the 85% v/v hexane and 15% v/v diethyl ether wherein steryl esters eluted at a distance almost equal to the solvent front (R_f ~1) while sitosterols remained close to the sample introduction point (R_f ~0). Lower R_f values were obtained when pure n-hexane was used as eluent. In SPE experimental design, n-hexane combined with solvents with similar polarities with diethyl ether were tested.

3.6 Solid-phase extraction

Five parallel extractions of 0.6 mL of the ~20 mg/mL (~12 mg) TOP 4 hexane-soluble fraction stock solution using a 100 mg Strata® Si-1 silica cartridges were combined for SPE analysis. Different elution solvent combinations of increasing polarity were used to extract the high MW siloxanes.

N-hexane combined with either MTBE, ethyl acetate, or DCM were tested to determine which solvent combination would result in the best extraction of the high MW siloxanes. The combination of n-hexane and DCM showed the most promising fractionation of a spiked TOP 4 hexane-soluble fraction as it collected the steryl esters and the high MW PDMS in one eluate, as shown in Figure 25. The results of n-hexane:MTBE and n-hexane: ethyl acetate are shown in Appendix B Figures B5 and B6.

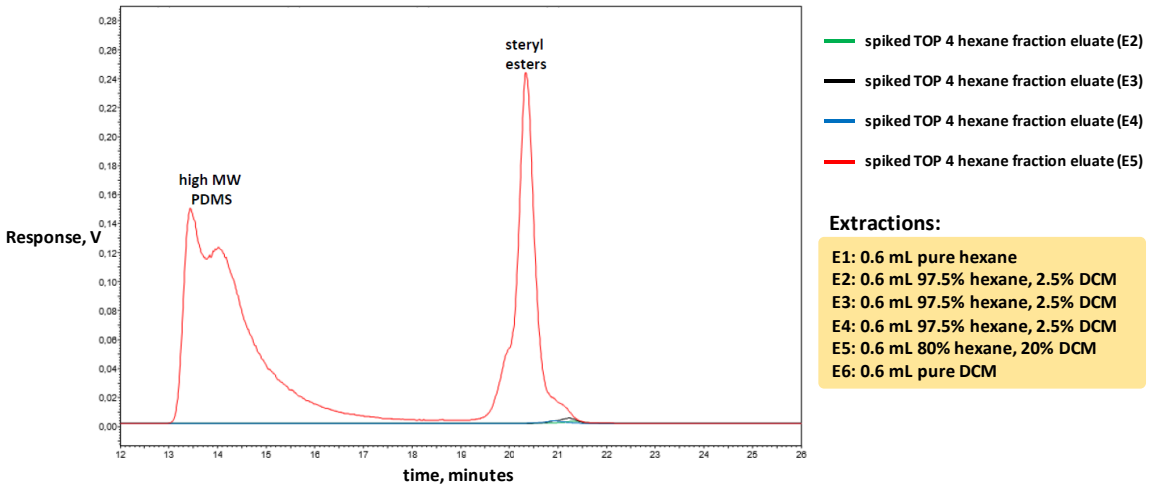


Figure 25. *HPSEC analysis result of spiked TOP 4 hexane-soluble fraction (2:1 v/v) using n-hexane:DCM elution solvents (Gain 3).*

Table 12 shows the elution solvent combinations of increasing polarity for the 100 mg Strata® Si-1 silica cartridges for SPE analysis. To see the effect of a slightly more polar solvent, the DCM content in the elution solvent is increased from 2.5% to 12.5%.

Table 12. *SPE elution solvent combinations for Strata® Si-1 silica cartridges*

Notation	Volume, mL	Description
E1	0.6	pure n-hexane
E2	**0.6**	**87.5% n-hexane, 12.5% DCM**
E3	0.6	87.5% n-hexane, 12.5% DCM
E4	0.6	87.5% n-hexane, 12.5% DCM
E5	0.6	80% n-hexane, 20% DCM
E6	0.6	pure DCM

Figure 26 shows the HPSEC analysis of the second eluate, E2, which registered a peak between the 0.012 mg/mL and 0.004 mg/mL silicone oil solutions in THF. The estimated concentration of the TOP 4 sample based on the linear interpolation of peak areas is 0.005 mg/mL, which is presented in Table 13.

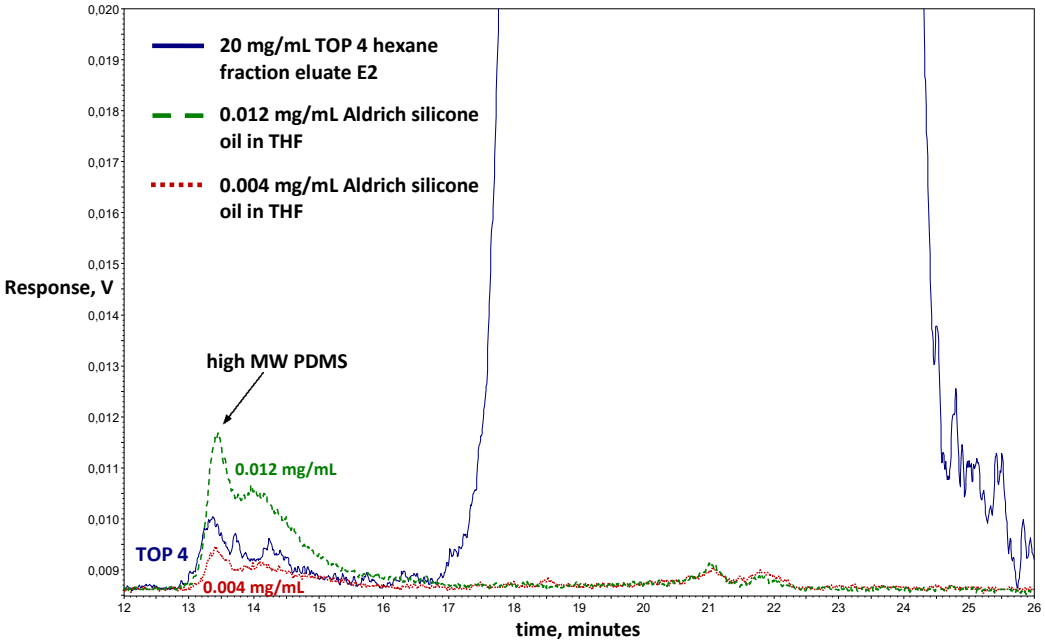


Figure 26. *HPSEC chromatograms post-SPE of five parallel extractions of 12 mg hexane-soluble TOP 4 sample compared to two Aldrich silicone oil standard solutions in THF with concentrations 0.004 mg/mL and 0.012 mg/mL (Gain 6).*

Table 13. *Total peak areas and concentrations of the TOP 4 hexane-soluble fraction and two Aldrich silicone oil solutions*

Sample	Total Peak Area	Concentration, µg/mL
0.004 mg/mL Aldrich silicone oil	50487	4
TOP 4 hexane-soluble fraction	**76801**	**5**
0.012 mg/mL Aldrich silicone oil	202758	12

The TOP peak for the PDMS region, 13-17 minutes, is quite different from the two standard Aldrich silicone oil solutions, which suggests that degradation of PDMS may have occurred during the processing of TOP. The HPSEC integration analysis results for the three samples are presented in Appendix B Figures B7 to B9, and the retention times and peak areas are shown in Appendix B Tables B2 to B4.

When loading ~12 mg of a sample or 12% of the bed capacity of the silica cartridge, breakthrough or early elution may occur since the recommended sample load for SPE is only around 1-5%. It is expected that the high MW siloxanes should elute at slightly more polar conditions than at 87.5% n-hexane and 12.5% DCM.

Table 14. *SPE elution solvent combinations for HyperSep SI cartridges*

Notation	Volume, mL	Description
E1	6	pure n-hexane
E2	6	87.5% n-hexane, 12.5% DCM
E3	6	87.5% n-hexane, 12.5% DCM
E4	6	87.5% n-hexane, 12.5% DCM
E5	6	87.5% n-hexane, 12.5% DCM
E6	6	85% n-hexane, 15% DCM
E7	6	80% n-hexane, 20% DCM
E8	6	60% n-hexane, 40% DCM
E9	6	pure DCM

Table 14 shows more solvent combinations for SPE using 1000 mg Thermo Scientific HyperSep SI cartridges. Spiking a hexane-soluble TOP 4 sample with

11% w/w Aldrich silicone oil and loading 3% of the total bed capacity to the HyperSep SI cartridge, most steryl esters were extracted in a series of 87.5% n-hexane, 12.5% DCM elution solvent (E2 to E5), and in 85% n-hexane, 15% DCM (E6), as seen in Figure 27.

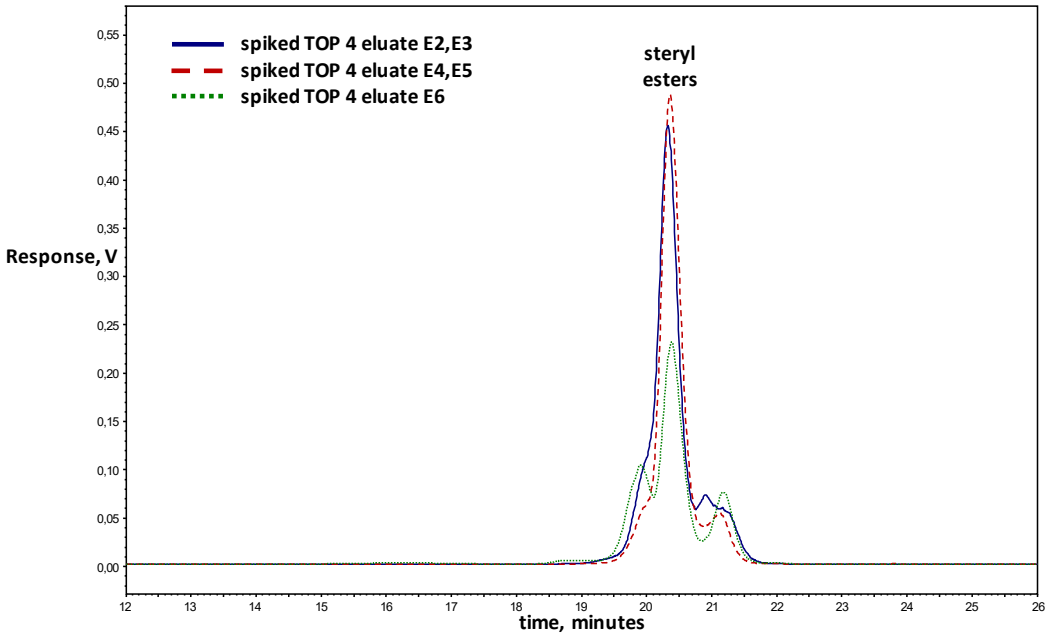


Figure 27. *HPSEC chromatograms post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 sample spiked with 11% w/w Aldrich silicone oil solution in n-hexane (eluates E2-E6, Gain 3).*

Figure 28 shows that the high MW siloxanes were collected using solvent combinations with at least 20% DCM (E7 to E9). In eluates E8 and E9, fatty and resin acids were predominant. This result confirms that the majority of the steryl esters can be separated from the high MW siloxanes. However, dimerised fatty acids, RT: 17.5-21.5 minutes, are still present in the desired eluates and must be separated by other means.

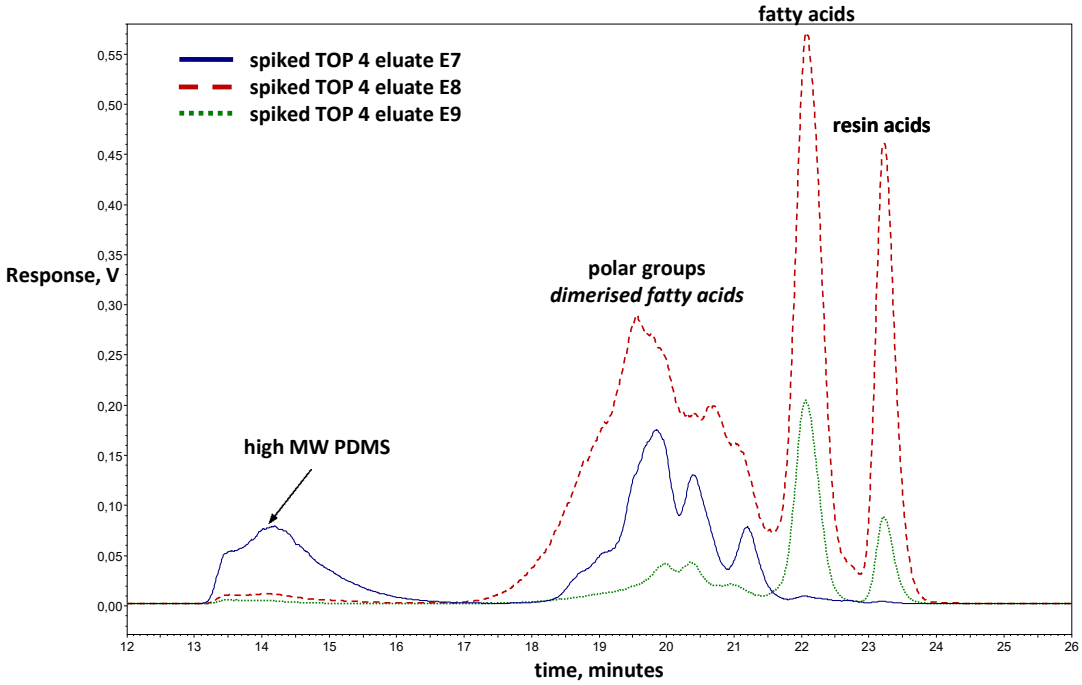


Figure 28. *HPSEC chromatograms post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 sample spiked with 11% w/w Aldrich silicone oil solution in n-hexane (eluates E7-E9, Gain 3).*

Dimerised fatty acids form when monoenoic acids (oleic and its isomers) and dieneoics react in the presence of an acidic clay catalyst [3,5] and when conjugated acids react via the Diels-Adler mechanism to form cyclic product [5].

Sithole and Filion [8] developed analytical procedures to separate silicone defoamer components from other pitch deposit components by solvent extraction and SPE. Silicone oil can be separated by performing sequential solvent extraction using acetone and chloroform for samples containing low MW PDMS (up to 10 kDa). Acetone extracts were loaded into a silica SPE cartridge (normal phase SPE) and the column is rinsed sequentially with elution solvents (hexane, chloroform, and methanol). The components in the chloroform fraction are further separated by loading into a C_{18}-silica SPE cartridge (reversed-phase SPE) using methanol and chloroform elution solvents. The silicone oil elutes in the chloroform fraction while wood resin elutes in the methanol fraction.

A short column GC-FID analysis of the SPE eluates of the 1.5 mL (~30 mg) spiked TOP 4 sample, as shown in Figure 29, confirmed that majority of steryl esters were

collected in a series of extraction using 87.5% n-hexane, 12.5% DCM elution solvent. The steryl esters content declined as the amount of DCM in the solvent was increased.

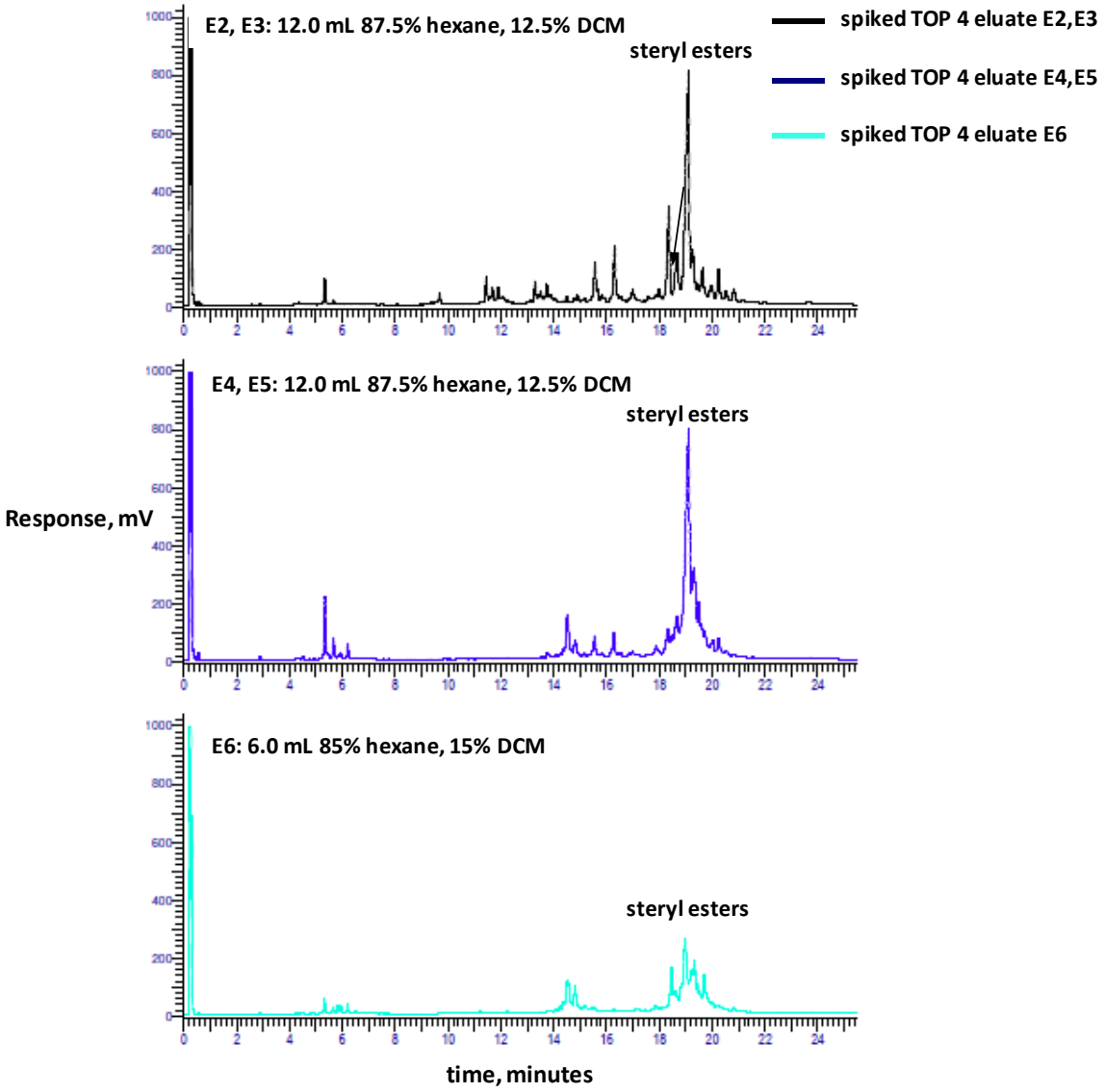


Figure 29. *Short-column GC-FID chromatograms post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 sample spiked with 11% w/w Aldrich silicone oil solution in n-hexane (eluates E2-E6).*

Fatty acids, resin acids, and sterols began to elute at 60% n-hexane, 40% DCM elution solvent, as seen in Figure 30. The high MW siloxanes eluted starting from E7 (80% n-hexane, 20% DCM elution solvent) based on the HPSEC results.

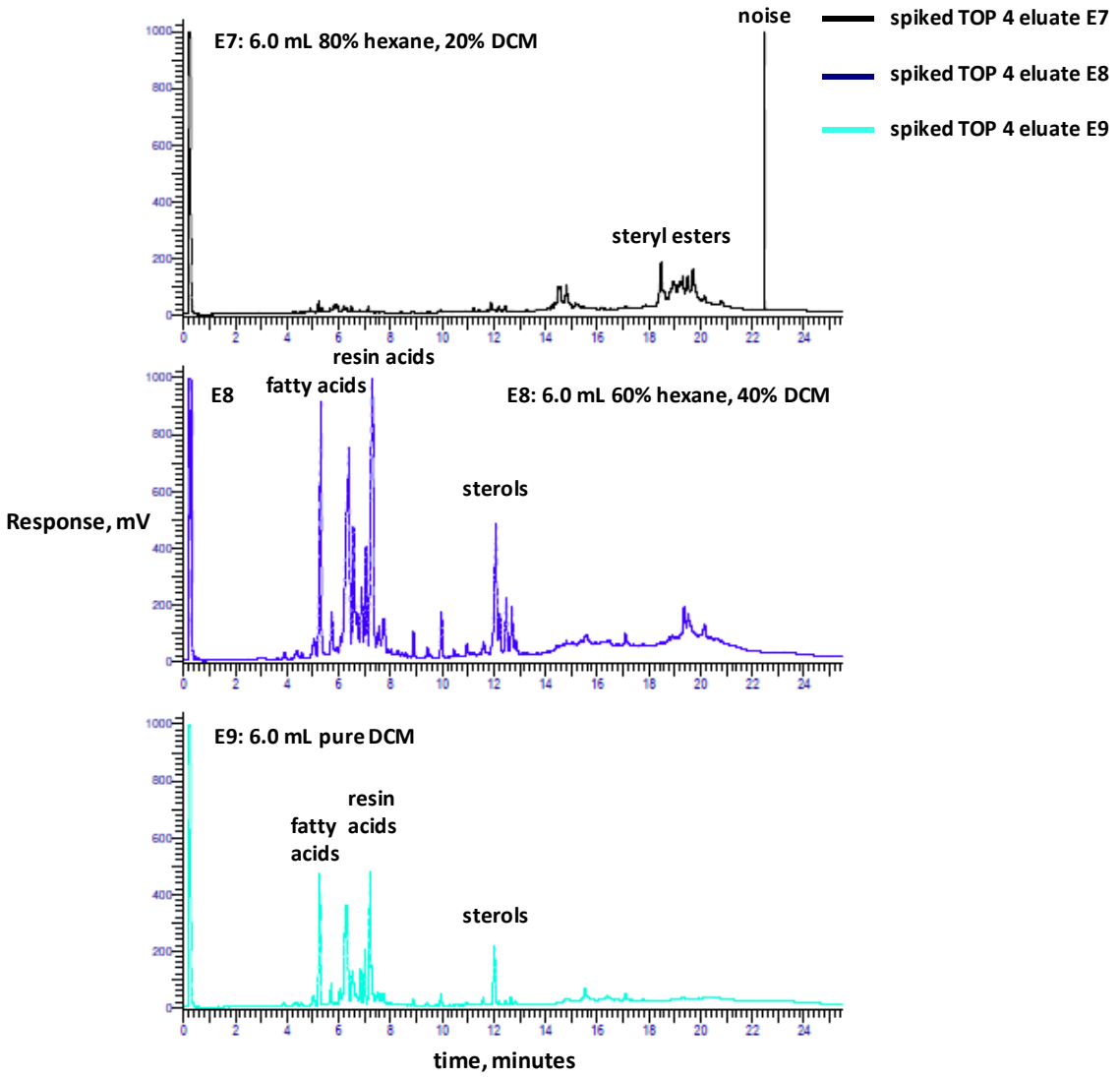


Figure 30. *Short-column GC-FID chromatograms post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 sample spiked with 11% w/w Aldrich silicone oil solution in n-hexane (eluates E7-E9).*

Different long-chain acid compounds were registered in SPE eluate 8 (6 mL of 60% n-hexane, 40% DCM), as shown in the GC-MS analysis in Figure 31. The most abundant acids were palmitic acid (acid 16:0, RT: ~20.3 min), linoleic acid (acid 18:2, RT: ~22.55 min), oleic acid (acid 18:1, RT: ~22.7 min), pimaric acid (RT: ~23.6 min), dehydroabietic acid (RT: ~24.55 min), and abietic acid (RT: ~25 min).

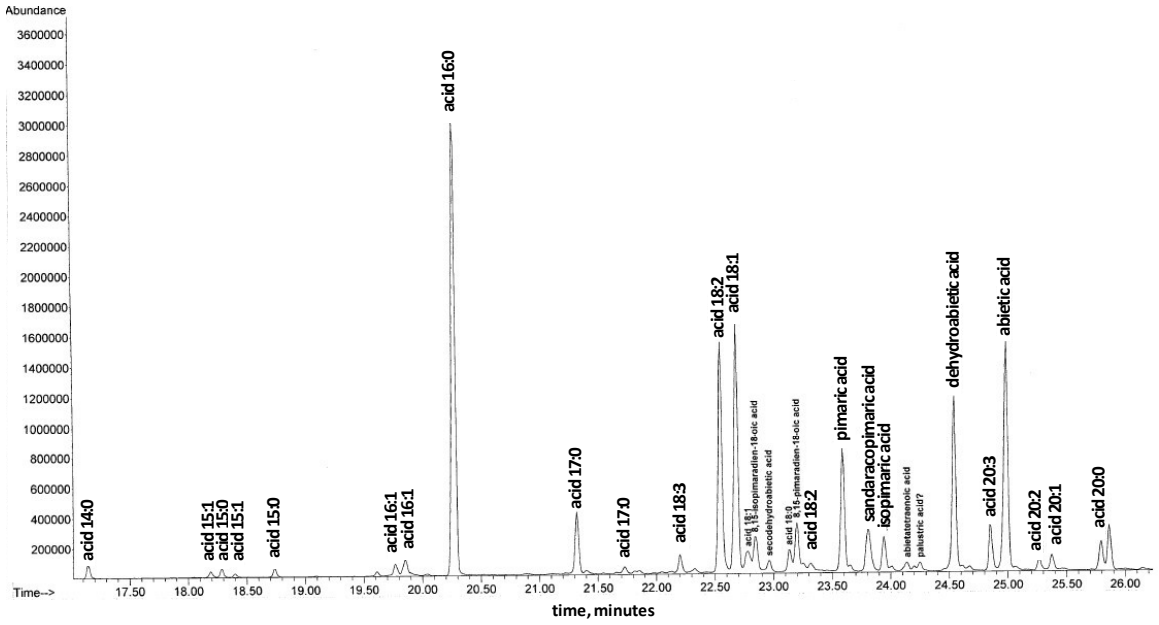


Figure 31. *GC-MS chromatogram post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 sample spiked with 11% w/w Aldrich silicone oil solution in n-hexane (eluate E8).*

The GC-MS chromatograms of eluates E2-E3 and E4-E5 are presented in Appendix C Figures C4 sand C5, respectively.

Figure 32 illustrates a comparison of the Aldrich silicone oil in n-hexane to a combination of eluates containing the high MW siloxanes. SPE of the Aldrich silicone oil solution showed that recovery was only 67% (based on peak areas) as the high MW PDMS components were not completely collected. Further optimisation or use of another cartridge may be necessary to improve the recovery.

The HPSEC integration analysis results for the Aldrich silicone oil in n-hexane with and without SPE are presented in Appendix B Figures B10 to B11, and the retention times and peak areas are shown in Appendix B Tables B5 to B6.

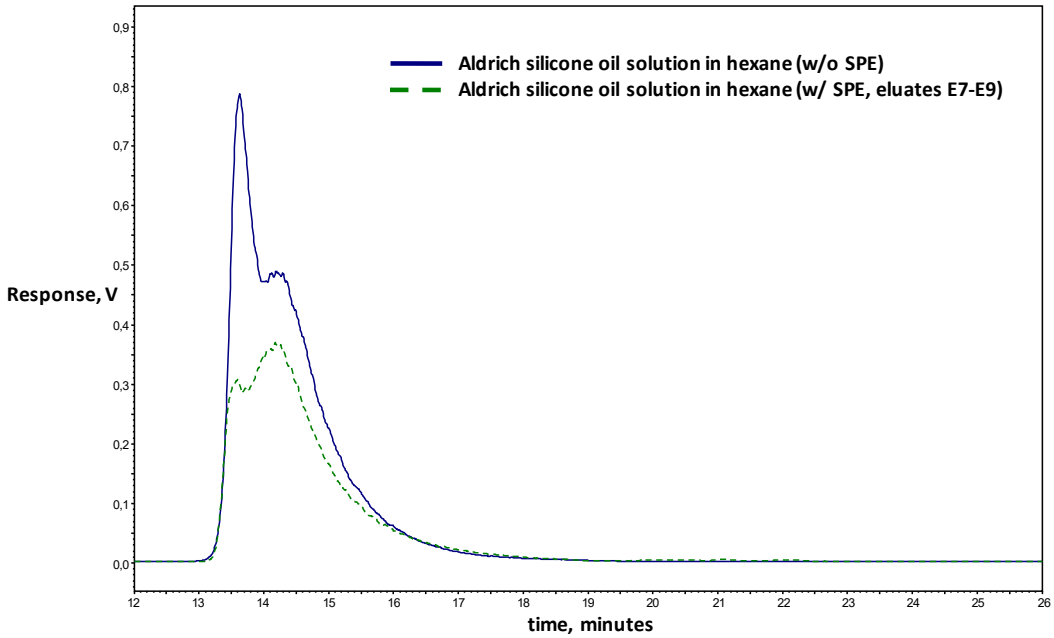


Figure 32. *HPSEC chromatograms of Aldrich silicone oil solution in hexane without SPE and eluates E7 to E9 of Aldrich silicone oil solution in n-hexane with SPE (Gain 3).*

3.7 ICP-MS

ICP-MS analyses were carried out to determine the total elemental Si concentrations in both the hexane-soluble and methanol-soluble TOP fractions. Results, as shown in Figure 33, reveal that ~90% of the total elemental Si was collected in the hexane-soluble fractions while <10% was transferred to the methanol-soluble fractions, with the exception of TOP 2 sample wherein the hexane-soluble fraction and the original sample concentrations were relatively equal which is caused by either some error in the extraction or in the preparation of the sample. Improvements in the extraction procedures must be carried out to isolate the Si compounds solely in the hexane-soluble fraction. The reference data for both the hexane- and methanol-soluble TOP fractions are presented in Appendix F Table F1.

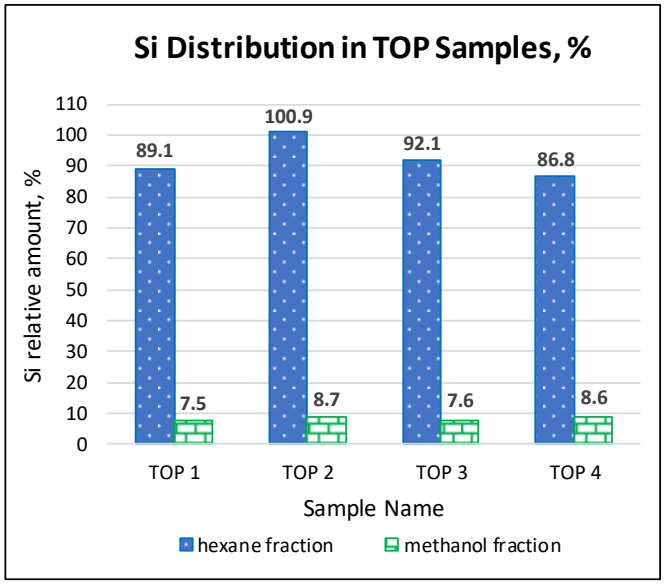


Figure 33. *ICP-MS analysis result of the Si distribution between the hexane-soluble and the methanol-soluble TOP fractions after performing the solvent extraction.*

Figure 34 illustrates the total elemental Si concentration levels in the four TOP samples using the single quadrupole Perkin Elmer SCIEX ELAN DRC^{PLUS} ICP-MS at standard mode (with and without HF addition), and an Agilent 8900 Triple Quad ICP-MS/MS using oxygen (O_2) reaction gas. The Si analysis of TOP samples using the Agilent 8900 Triple Quad was performed by an external laboratory, and the measurement specifications are shown in Appendix F Table F2.

The calibration curve figures and tables for 10 to 200 ppb Si standard solutions with (Calibration A) and without (Calibration B) addition of HF acid are presented in Appendix F (Figures F1 and F2, and Table F3).

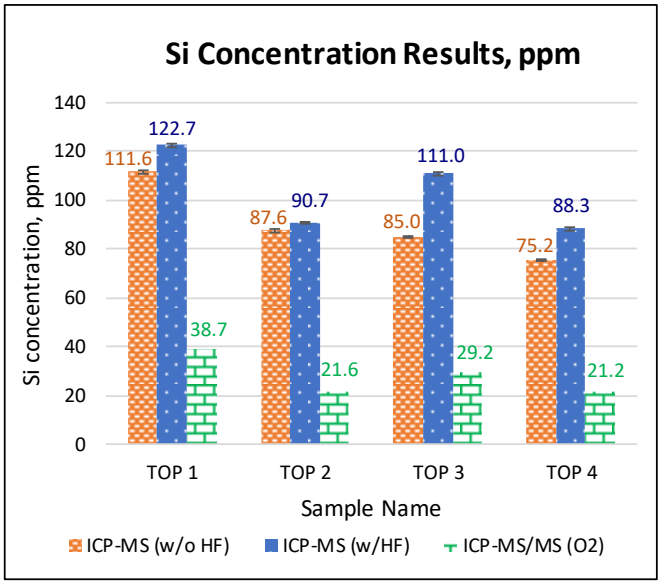


Figure 34. *ICP-MS analysis comparison of the results obtained by a single quadrupole ICP-MS (with and without HF acid addition) at standard mode, and an ICP-MS/MS using O_2 reaction gas.*

The Si concentrations ranged between 75.2 and 111.6 ppm using a single quadrupole instrument without HF addition, while the concentrations ranged between 88.3 and 122.7 ppm when 10 µL HF was added during microwave digestion. This slight increase in Si concentration in the TOP samples may have been caused using a glass nebuliser and spray chamber, and a quartz injector. These components are also made of Si-containing compounds, and Si leaching out from these components is possible. The numerical values of the Si concentrations of the four TOP samples including blank solutions are shown in Appendix F Table F4.

Analysing the same set of samples using an ICP-MS/MS instrument and employing O_2 as the reaction gas, the Si concentrations were only between 21.2 and 38.7 ppm. The significant disparity in Si concentrations is attributed to the difference in ICP-MS analysis mode. The typical standard mode is more prone to nitrogen based ($^{14}N_2^+$) and carbon plus oxygen based ($^{12}C^{16}O^+$) polyatomic interferences. These ions have the same mass-to-charge ratio *(m/z)* as Si at 28 and likely originate from residual digestion products. These interferences were however resolved when O_2 reaction gas was employed and Si was quantified as the reaction product at *m/z* 44 (SiO^+) instead of *m/z* 28. As $^{14}N_2^+$ and $^{12}C^{16}O^+$ do not produce reaction products

with oxygen at mass 44 the analysis is free from these interferences and hence, lower Si concentrations were determined.

3.8 Proposed analytical scheme

Considering all relevant results obtained from solvent extraction to ICP-MS analysis, Figure 35 depicts the proposed analytical procedure for the analysis of silicone oil in tall oil products.

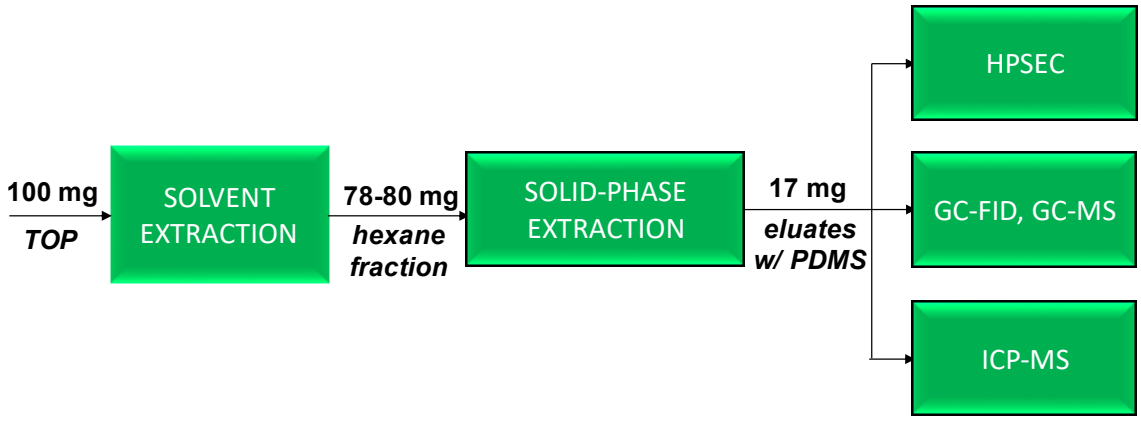


Figure 35. *Proposed scheme for the analysis of silicone oil in tall oil products showing different analytical techniques with corresponding material balance*

The tall oil product sample components were initially separated by solvent extraction using n-hexane and methanol. The hexane-soluble fraction components were further fractionated by SPE using silica cartridges (normal phase SPE). The desired eluates, E7-E9, were combined for HPSEC, GC-FID, GC-MS, and ICP-MS analyses.

Gravimetric analysis of the different fractions shows that given a 100 mg TOP sample, 78-80 mg or 78-80% of its total mass is extracted in the hexane-soluble fraction. Loading the hexane-soluble fractions into silica cartridges, the desired SPE eluates contain only 17 mg or 17% of the original TOP sample by mass. As the SPE recovery value relatively low at ~67%, as shown in Figure 32, it is advisable to improve the SPE conditions to collect all high MW PDMS in one or several fractions.

4. SUMMARY AND CONCLUSIONS

Analytical procedures were developed to concentrate the high MW PDMS in tall oil products using several analytical techniques. The TOP samples were initially fractionated by solvent extraction using n-hexane and methanol. By gravimetric determination, the hexane-soluble fractions were 77.7-80.5% by mass while only 19.5-22.3% were transferred to the methanol-soluble fractions. ICP-MS measurements of the total Si concentration in the hexane- and methanol-soluble TOP fractions revealed that ~90% of the total elemental Si was collected in the hexane-soluble fraction while only <10% was retained in the methanol-soluble fraction.

HPSEC and GC-FID analyses of the TOP samples and their corresponding hexane- and methanol-soluble fractions confirmed the presence of steryl esters, sterols, fatty, and resin acids in all TOP samples, with slight variation in fatty and resin acids content. Majority of the steryl esters were extracted in the hexane-soluble fraction while the fatty acids, sterols, and resin acids were distributed between the two fractions.

The HPSEC analysis of a TOP 4 sample spiked with 50 mg of Aldrich silicone oil solution in n-hexane, revealed that the high MW PDMS was collected in the hexane-soluble fraction together with steryl esters. These results established the preliminary separation of steryl esters and the high MW PDMS from sterols, fatty and resin acids by solvent extraction.

The results of the initial separation of TOP components by solvent extraction using n-hexane and methanol were acceptable since the high MW PDMS were collected only in the hexane-soluble fraction, but since the fatty and resin acids were distributed between the two fractions, investigating other solvent combinations or improving the extraction conditions can be carried out to isolate the acidic components entirely into the methanol-soluble fraction.

HPSEC and GC-FID analyses of the hexane-soluble fractions of CTO 1 and the four process samples (P1 to P4) showed that the P1, P4, and CTO 1 samples were

comprised mainly of fatty and resin acids, the P2 sample was predominantly fatty acids, and the P3 sample closely resembled the TOP samples.

HPSEC/GPC analysis based on the calibration curve of polystyrene of 1 mg/mL of both the antifoam emulsion hexane-soluble fraction and the Aldrich silicone oil showed comparable molecular weights with the antifoam emulsion having a weight average molecular weight of 48 kDa while the Aldrich silicone oil is 38 kDa. The Aldrich silicone oil was used as the source of high MW siloxanes in spiking the TOP samples as it did not contain the low MW components which were present in the antifoam emulsion.

The alkaline hydrolysis of the TOP 4 hexane-soluble fraction using 0.5 M KOH in 90% ethanol resulted in the hydrolysis of steryl esters to free sterols, primarily sitosterol, while the fatty and resin acid components remained in the alkali-water phase. The HPSEC comparison of a hydrolysed hexane-soluble TOP 4 sample fraction against an unhydrolysed fraction resulted in longer retention times, which was a desirable situation as the hydrolysed components elute much later than the high MW PDMS.

However, the high MW siloxanes in the Aldrich silicone oil were also hydrolysed into low MW siloxanes or linear and cyclic oligomers. The hydrolysis of the Aldrich silicone oil confirmed that the concentration of the alkali added was relatively strong as it hydrolysed both the TOP sample components and the Aldrich silicone oil. Alkaline hydrolysis is a promising technique in eliminating silicone oil in TOP samples, but if identification and quantification of high MW PDMS are preferred, then it is advisable to have slightly less alkaline conditions than the 0.5M KOH in 90% ethanol to prevent PDMS hydrolysis.

The SPE analysis of the five parallel extractions of 0.6 mL of the ~20 mg/mL (~12 mg) TOP 4 hexane-soluble fraction using a 100 mg Strata® Si-1 silica cartridges registered a peak between the 0.012 mg/mL and 0.004 mg/mL Aldrich silicone oil solutions in THF, and its estimated concentration based on the linear interpolation of peak areas is 0.005 mg/mL.

Testing different SPE solvent combinations using 1000 mg Thermo Scientific HyperSep SI cartridges and spiking the TOP 4 hexane-soluble sample with 11% w/w Aldrich silicone oil resulted in the extraction of most steryl esters in a series of 87.5% n-hexane, 12.5% DCM elution solvent (E2 to E5), and in 85% n-hexane, 15% DCM (E6). The high MW siloxanes were collected together with some fatty and resin acids by using solvent combinations with at least 20% DCM (E7 to E9). These results confirmed that the majority of the steryl esters were separated from the high MW siloxanes, however, dimerised fatty acids, RT: 17.5-21.5 minutes, were still present in the desired eluates and these compounds interfere with the tail of the PDMS peak by HPSEC analysis.

The SPE comparison of the Aldrich silicone oil in n-hexane to a combination of eluates containing the high MW siloxanes (E7-E9) showed that recovery was only 67% based on peak areas as the high MW PDMS components were not completely collected. This low recovery value indicates that some of the high MW PDMS remained in the SPE cartridge requiring further optimisation.

The ICP-MS analysis of the TOP samples using a single quadrupole instrument at standard mode obtained Si concentrations between 75.2 and 122.7 ppm which were much higher than the 21.2 to 38.7 ppm Si as analysed by an ICP-MS/MS instrument with O_2 as the reaction gas. The $^{14}N_2^+$ and $^{12}C^{16}O^+$ interferences on mass 28, which were present in the standard mode, were resolved by the use of O_2 reaction gas by measuring Si at *m/z* 44 (SiO^+) instead of *m/z* 28, hence, lower and more representative Si concentrations were achieved with this approach.

Overall, the proposed analytical scheme for the analysis of silicone oil in tall oil, as shown in Figure 35, is a good starting point to concentrate the high MW PDMS present in tall oil products. Since the developed procedures were only tested on TOP samples, it is necessary to evaluate the procedures using other samples to prove if it applies to all tall oil products.

5. RECOMMENDATIONS

To further improve the separation of the high MW PDMS in tall oil products, it is recommended to enhance the phase separation of steryl esters from fatty acids, sterols, and resin acids. Lowering the amount of sample weighed instead of 250 mg and extracting using the same solvent volumes or by making the solution slightly alkaline to transfer all the acidic components to the methanol phase without experiencing hydrolysis of the high MW PDMS could improve the phase separation of tall oil components.

Another recommendation is to evaluate the possibility of using other preparative separation technique (e.g., HPLC) prior to SPE to eliminate overlapping of peaks at RT: 16-17 minutes in HPSEC. Testing of other SPE cartridges aside from the silica cartridges such as SiOH, Florisil®, NH_2, CN, and OH by investigating normal-phase followed by reversed-phase SPE on the tall oil product samples may also be done to improve the separation of the high MW PDMS.

Once the separation of the high MW PDMS in TOP is optimised, the total elemental Si concentrations of desired SPE eluates must be analysed by ICP-MS/MS using O_2 as the reaction gas to obtain reliable Si concentration values. It would also be recommended to test the high MW PDMS sources, the Aldrich silicone oil and the antifoam emulsion, for ICP-MS analysis.

Since the developed analytical procedures were only tested for the TOP samples, it is advisable to perform the developed procedures also to CTO 1, and the process samples P1-P4. Validation of the procedures must also be carried out to determine relevant performance characteristics (e.g., repeatability, detection limits, quantitation limits, linearity, and recovery).

Reactor experiments on the antifoam emulsion and thermogravimetric analysis of the TOP samples can be carried out in future experimental runs to determine reaction mechanisms and the performance of silicones at high or low temperatures by simulating the industrial pulping conditions.

6. REFERENCES

[1] Jong, E. d., Jungmeier, G. (2015). Chapter 1 - Biorefinery Concepts in Comparison to Petrochemical Refineries. In *Industrial Biorefineries and White Biotechnology (pp. 3-33)*. Elsevier B.V. http://dx.doi.org/10.1016/B978-0-444-63453-5.00001-X.

[2] Jungmeier G, Hingsamer M, van Ree R. (2013). *Biofuel-driven biorefineries. A selection of the most promising biorefinery concepts to produce large volumes of road transportation biofuels by 2025*. IEA Bioenergy – Task 42 Biorefinery.

[3] Drew, J., Propst, M. (1981). *Tall oil: A book on the processing and use of tall oil; for chemists, engineers, managers and producers.* New York: Pulp Chemicals Association.

[4] Wansbrough, H. (1998). Tall Oil Production and Processing. In J. R. John, E. Packer, *Chemical Processes in New Zealand.* New Zealand Institute of Chemistry.

[5] McSweeney, E.E., Arlt, H. G., Jr., Russell, J. (1987). *Tall Oil and Its Uses - II.* New York: Pulp Chemicals Association, Inc.

[6] Sundberg, A. (2017). *Wood and Paper Chemistry 423102.0.* Turku: Åbo Akademi University.

[7] Holmbom, B. and Erä, V. (1978). Composition of tall oil pitch. *Journal of Oil & Fat Industries*. 55. 342-344. 10.1007/BF02669926.

[8] Sithole, B., Filion, R. (2004). Determination of silicone defoamers in mill pitch deposits. *African Pulp and Paper Week 2004*. Pointe-Claire, QC: Pulp and Paper Research Institute of Canada.

[9] Chao, S. (2009). Silicones in the Pulp and Paper Industry. *Silicones in Industrial Applications* (pp. 16-18). Dow Corning Corporation.

[10] Graiver, D, Farminer, K.W., and Narayan, R. (2003). A Review of the Fate and Effects of Silicones in the Environment. *Journal of Polymers and the Environment*, 129-136.

[11] Colas, A. (2009). Introduction to Silicone Chemistr. *Silicones in Industrial Applications* (pp. 2-5). Dow Corning Corporation.

[12] Kupareva, A., Mäki-Arvela, P., Grénman, H., Eränen, K., Hemming, J., and Murzin, D. Y. (2015). The transformation of silicon species contained in used oils under industrially relevant alkali treatment conditions. *J. Chem. Technol. Biotechnol.*, 90: 1991-1998.

[13] Rydberg, J. (2004). *Solvent extraction principles and practice* (2. ed., rev. and expanded.). New York: Marcel Dekker.

[14] Bele, A., and Khale, A. (2011). An overview on thin layer chromatography. *International Journal of Pharmaceutical Sciences and Research*. Vol. 2(2): 256-267.

[15] Panagiotopoulou, P. M., and Tsimidou, M. (2002). Solid phase extraction: Applications to the chromatographic analysis of vegetable oils and fats. Grasas y Aceites. 53. 10.3989/gya.2002.v53.i1.292.

[16] Guide to Solid Phase Extraction (1998). Bellefonte, PA: Supelco (Sigma-Aldrich Co.).

[17] Dupont, A. (2009). Characterization of Silicones. *Silicones in Industrial Applications* (pp. 5-11). Dow Corning Corporation.

[18] SEDEX Model 85LT Low Temperature Evaporative Light Scattering Detector Operator's Manual. (2012). SEDERE, S.A.S.

[19] The 30-Minute Guide to ICP-MS. (2004). Waltham, Massachusetts, USA: Perkin Elmer, Inc.

7. APPENDICES

Appendix A. Solvent extraction

Table A1. *Selected properties of n-hexane, methanol, and water [13]*

Solvent	Molar mass (M), g/mol	Molar volume (V^b), mL/mol	Dipole moment (μ^c) D	Dielectric constant ε^b	δ $(J/mL)^{1/2}$
n-hexane	86.2	131.6	0.09	1.88	15.0
water	18.0	18.1	1.85	78.36	47.9[d]
methanol	32.0	40.7	2.87	32.66	29.3

[b]At 25 °C

[c]Isolated solvent molecules, i.e., in the gaseous phase or dilute solution in an inert solvent

[d]Behaves in organic-rich aqueous mixtures as if δ ≈ 30.

Debye unit (D) = 3.336×10^{-30} C · m

Table A2. *Mass distribution of the hexane- and methanol-soluble fractions of the four TOP samples post-solvent extraction*

Sample	mass of sample, mg	mass of test tube, mg	mass of test tube + sample, mg	mass of extracted hexane-soluble fraction, mg	extracted hexane-soluble fraction, %	mass of extracted methanol-soluble fraction[a], mg	extracted methanol-soluble fraction, %	
TOP 1	308.58	10941.67	11186.11	244.44	79.21	64.14	20.79	
	251.98	11458.67	11655.64	196.97	78.17	55.01	21.83	
	242.90	10853.57	11041.12	187.55	77.21	55.35	22.79	
	245.11	10910.81	11098.97	188.16	76.77	56.95	23.23	
	252.40	10817.41	11013.41	196.00	77.65	56.40	22.35	
SUM	**1300.97**				**77.80**		**22.20**	**AVE**
TOP 2	262.19	11045.23	11248.09	202.86	77.37	59.33	22.63	
	257.72	10918.02	11117.61	199.59	77.44	58.13	22.56	
	255.02	10830.14	11029.08	198.94	78.01	56.08	21.99	
	286.72	10843.25	11065.78	222.53	77.61	64.19	22.39	
	246.69	10884.94	11077.92	192.98	78.23	53.71	21.77	
SUM	**1308.34**				**77.73**		**22.27**	**AVE**
TOP 3	245.92	11083.32	11278.22	194.90	79.25	51.02	20.75	
	247.94	10914.03	11109.26	195.23	78.74	52.71	21.26	
	274.24	10925.79	11145.06	219.27	79.96	54.97	20.04	
	293.48	10831.35	11064.43	233.08	79.42	60.40	20.58	
	259.70	10863.92	11069.43	205.51	79.13	54.19	20.87	
SUM	**1321.28**				**79.30**		**20.70**	**AVE**
TOP 4	265.89	10991.59	11205.52	213.93	80.46	51.96	19.54	
	268.92	11398.69	11616.57	217.88	81.02	51.04	18.98	
	264.02	10882.26	11095.32	213.06	80.70	50.96	19.30	
	255.02	11347.37	11553.01	205.64	80.64	49.38	19.36	
	255.75	11017.26	11221.25	203.99	79.76	51.76	20.24	
SUM	**1309.60**				**80.52**		**19.48**	**AVE**
				AVERAGE	**78.84**		**21.16**	

[a]Mass difference of the sample and the extracted hexane-soluble fraction

Table A3. *Average concentrations of the stock solution of hexane-soluble fractions of the four TOP samples*

Sample	mass of test tube, mg	mass of test tube + sample, mg	mass of hexane-soluble fraction, mg	concentration of hexane-soluble fraction, mg/mL	average concentration of hexane-soluble fraction, mg/mL
TOP 1	11774.04	11793.78	19.74	19.74	**19.89**
	10980.14	11000.22	20.08	20.08	
	10827.86	10847.67	19.81	19.81	
	10716.73	10736.72	19.99	19.99	
	10928.68	10948.52	19.84	19.84	
TOP 2	10856.35	10876.07	19.72	19.72	**19.75**
	10898.13	10917.80	19.67	19.67	
	11573.87	11593.48	19.61	19.61	
	12822.82	12842.60	19.78	19.78	
	10700.38	10720.36	19.98	19.98	
TOP 3	11565.61	11585.30	19.69	19.69	**19.72**
	10942.62	10962.40	19.78	19.78	
	12580.85	12600.64	19.79	19.79	
	11543.30	11562.83	19.53	19.53	
	10958.56	10978.35	19.79	19.79	
TOP 4	11340.93	11361.28	20.35	20.35	**20.29**
	10976.93	10997.20	20.27	20.27	
	10870.83	10891.04	20.21	20.21	
	11660.49	11680.83	20.34	20.34	
	12773.18	12793.44	20.26	20.26	

Appendix B. HPSEC results

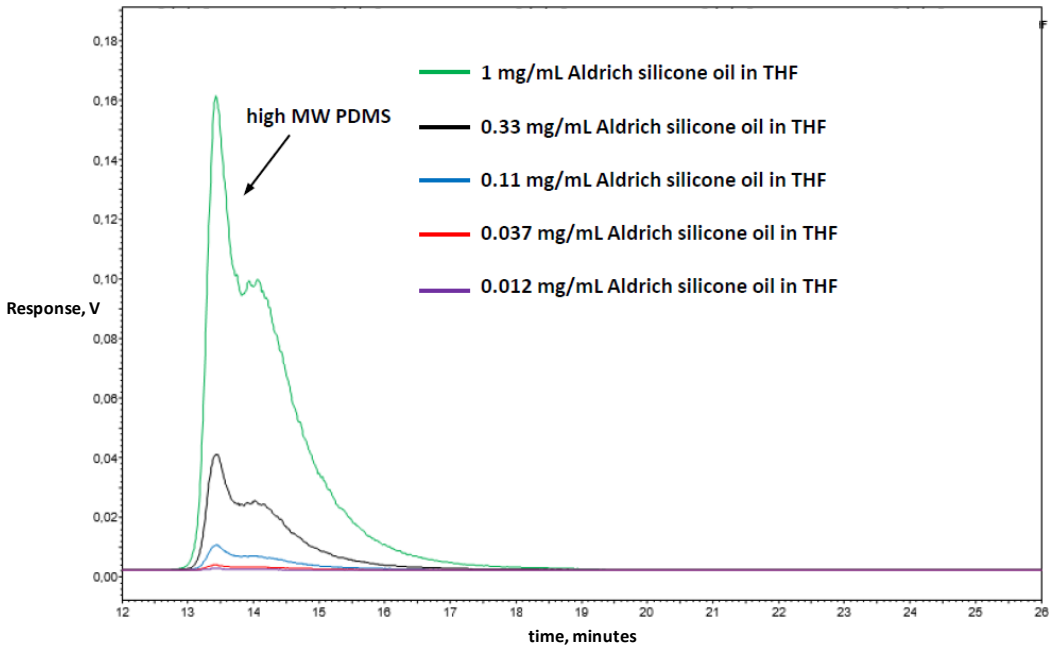


Figure B1.1. *HPSEC analysis result of the sensitivity test of different concentrations of Aldrich silicone oil in THF solutions (Gain 3).*

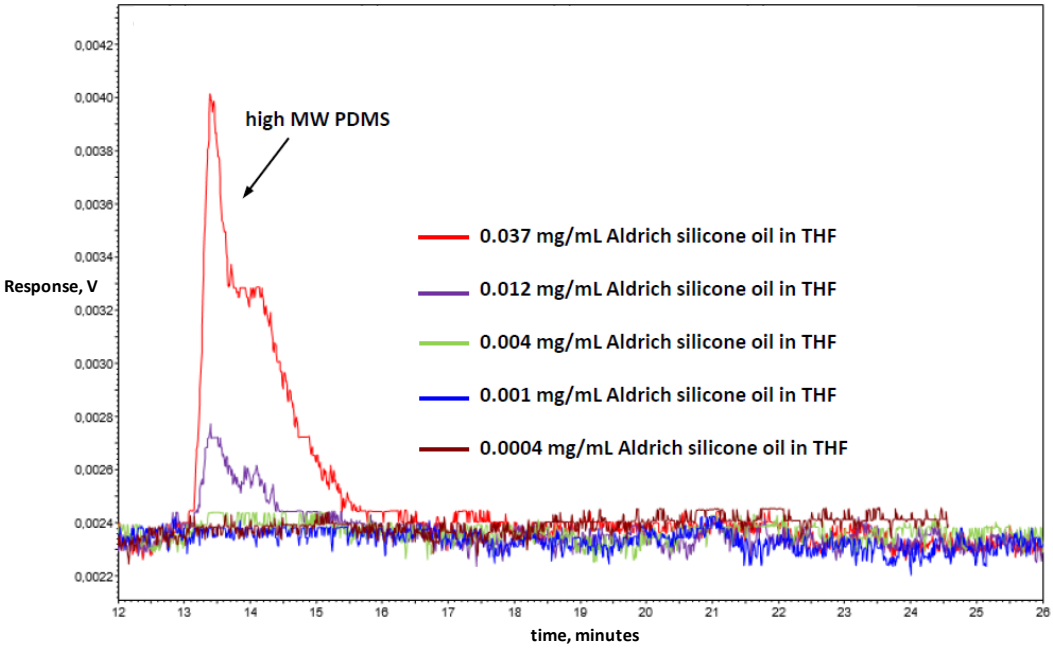


Figure B1.2. *HPSEC analysis result of the sensitivity test of different concentrations of Aldrich silicone oil in THF solutions (Gain 3).*

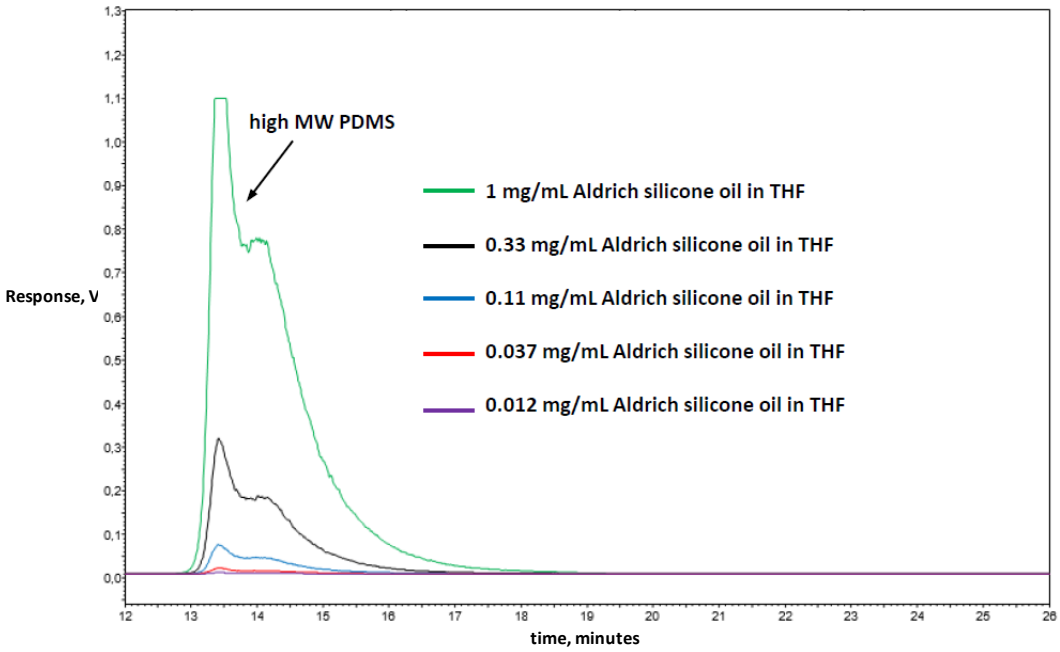


Figure B2.1. *HPSEC analysis result of the sensitivity test of different concentrations of Aldrich silicone oil in THF solutions (Gain 6).*

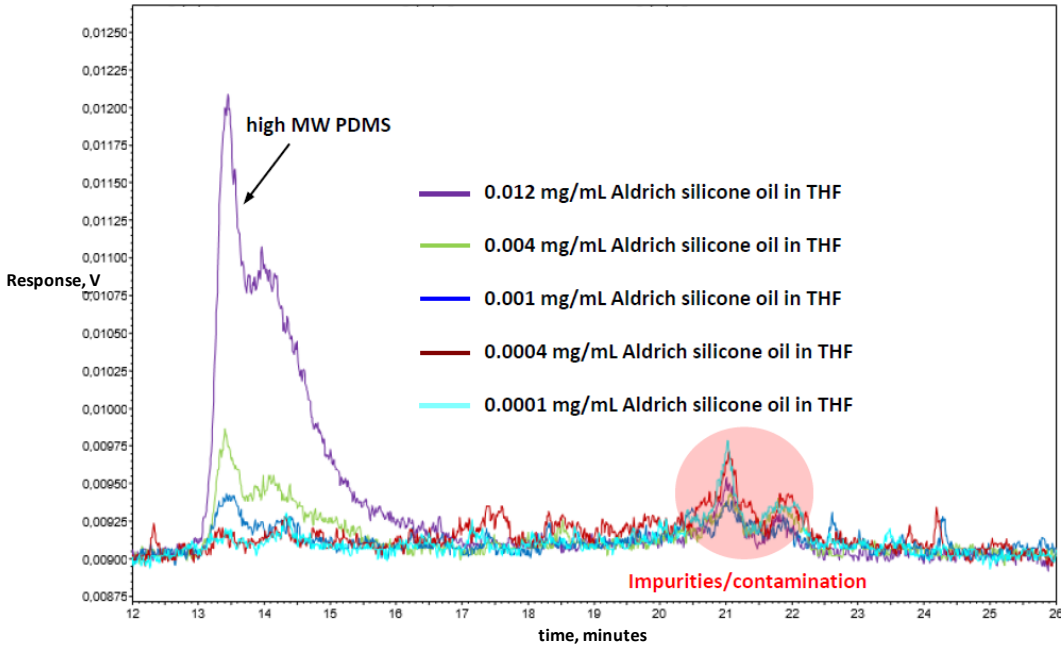


Figure B2.2. *HPSEC analysis result of the sensitivity test of different concentrations of Aldrich silicone oil in THF solutions (Gain 6).*

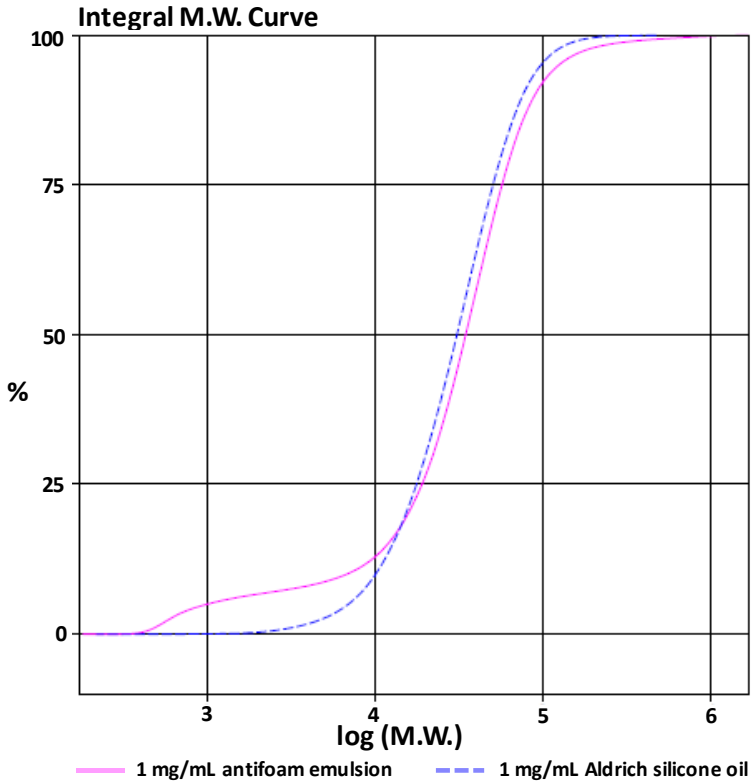


Figure B3. *Integral molecular weight curves of 1 mg/mL antifoam emulsion hexane-soluble fraction and 1 mg/mL Aldrich silicone oil solution in THF.*

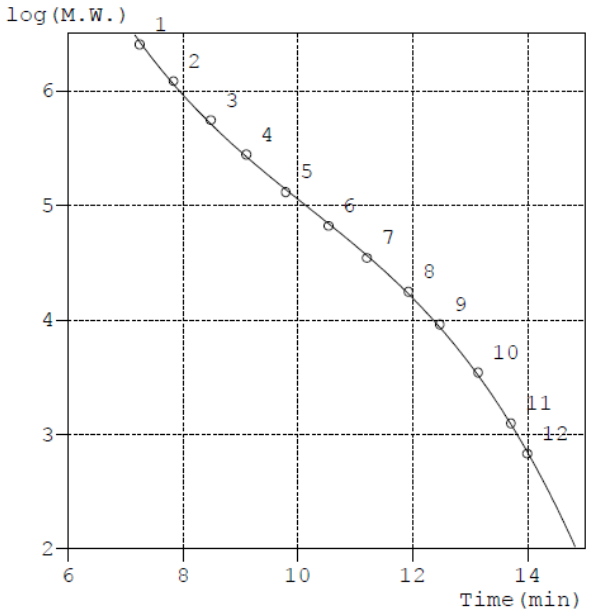


Figure B4. *Calibration curve of polystyrene standard for the HPSEC/GPC analysis of the antifoam emulsion hexane-soluble fraction and 1 mg/mL Aldrich silicone oil solution in THF.*

Table B1. *Calibration curve table for the polystyrene standard (12 calibration points).*

Calibration Point	R.T. (min)	Molecular Weight (Da)
1	7.245	2,520,000
2	7.831	1,210,000
3	8.485	552,000
4	9.101	277,000
5	9.788	130,000
6	10.535	66,000
7	11.207	34,800
8	11.928	17,600
9	12.475	9,130
10	13.140	3,470
11	13.715	1,250
12	14.000	682

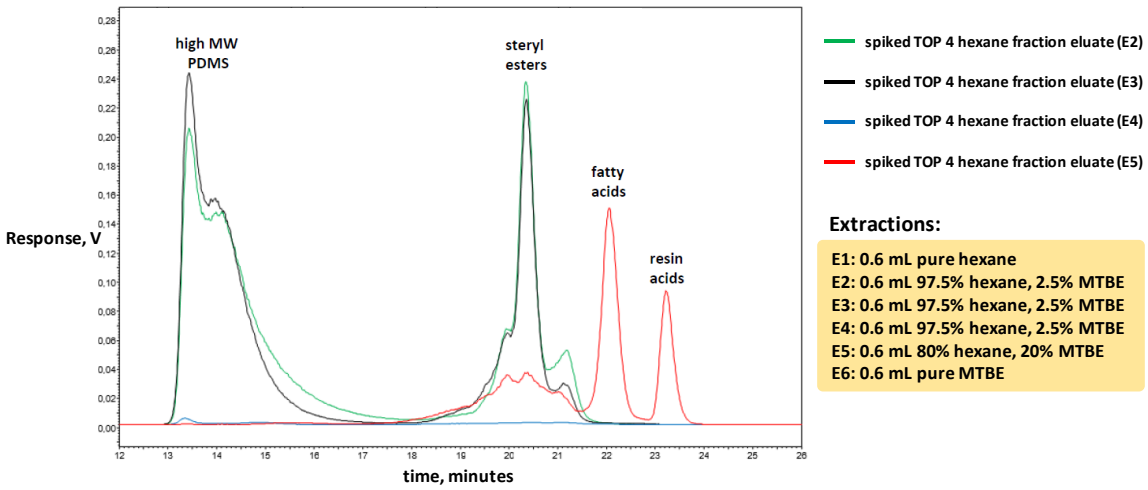


Figure B5. *HPSEC analysis result of spiked TOP 4 hexane-soluble fraction (2:1 v/v) using n-hexane:MTBE elution solvents (Gain 3).*

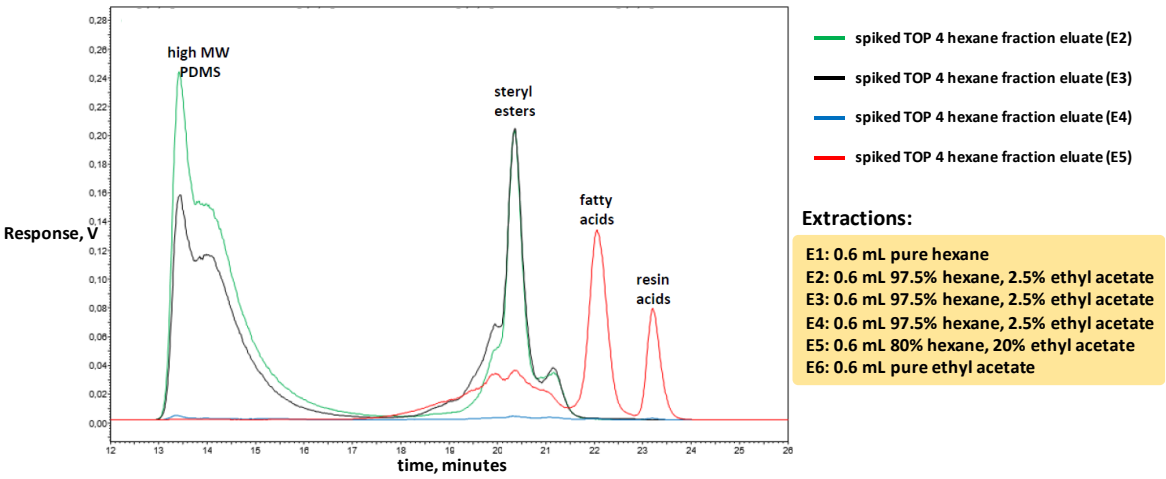


Figure B6. *HPSEC analysis result of spiked TOP 4 hexane-soluble sample fraction (2:1 v/v) using n-hexane:ethyl acetate elution solvents (Gain 3).*

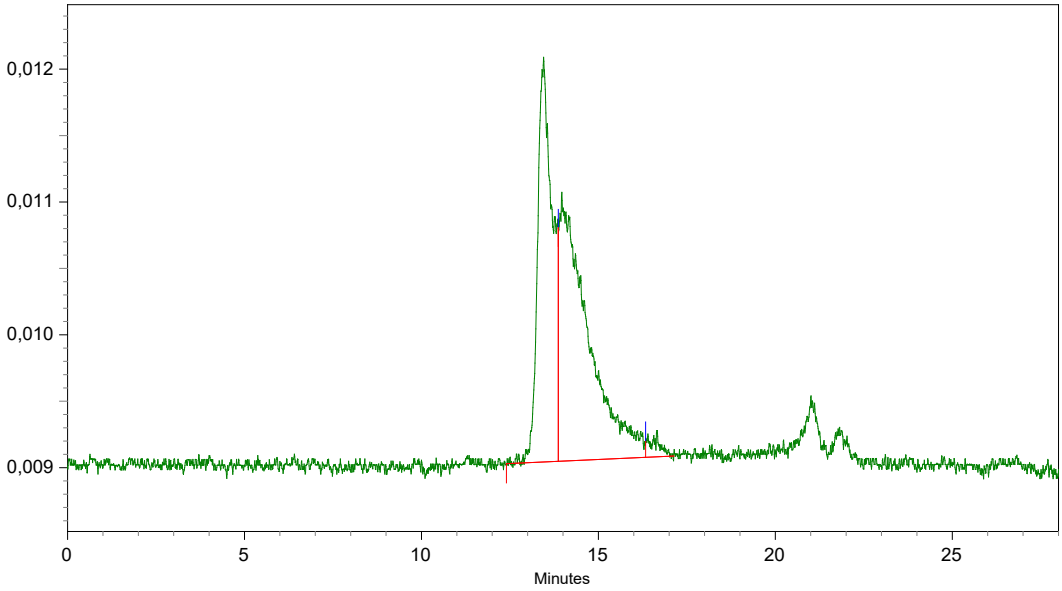


Figure B7. *HPSEC integration analysis result of the 0.012 mg/mL Aldrich silicone oil in THF solution (Gain 6).*

Table B2. *HPSEC integration analysis retention times and their corresponding peak areas for the 0.012 mg/mL Aldrich silicone oil in THF solution (Gain 6).*

Peak No.	Retention time, min	Area[a]	Area Percent
1	13.43	89397	44.1
2	14.00	113361	55.9
Total		**202758**	**100.0**

[a]Integration events parameters: width: 40, slope: 190, min. area: 10000

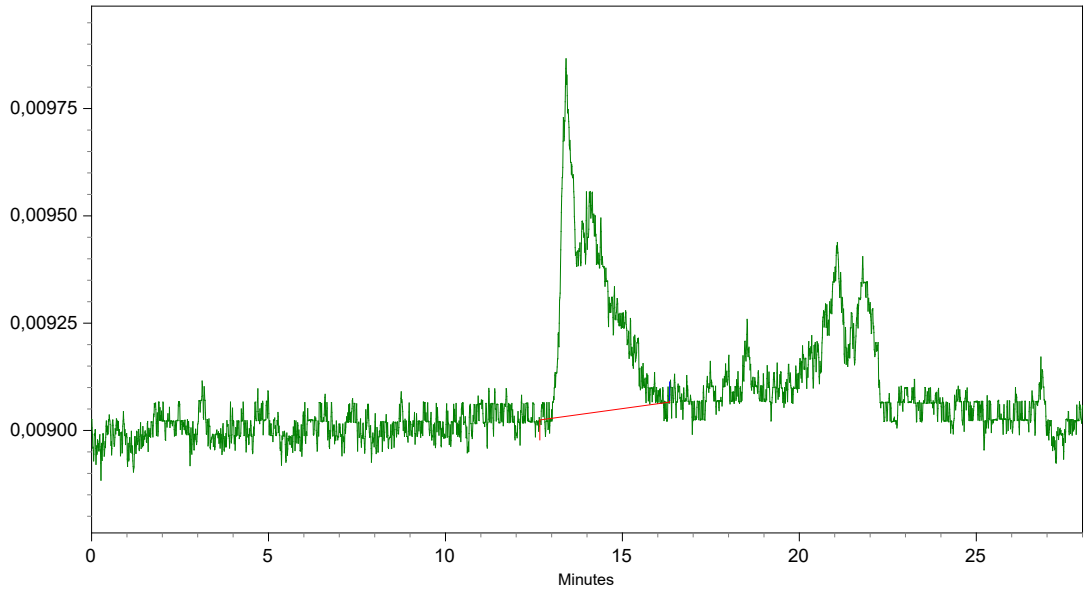


Figure B8. *HPSEC integration analysis result of the 0.004 mg/mL Aldrich silicone oil in THF solution (Gain 6).*

Table B3. *HPSEC integration analysis retention time and its corresponding peak area for the 0.004 mg/mL Aldrich silicone oil in THF solution (Gain 6).*

Peak No.	Retention time, min	Area[a]	Area Percent
1	13.55	50487	100.0
Total		**50487**	**100.0**

[a]Integration events parameters: width: 200, slope: 10, min. area: 3000

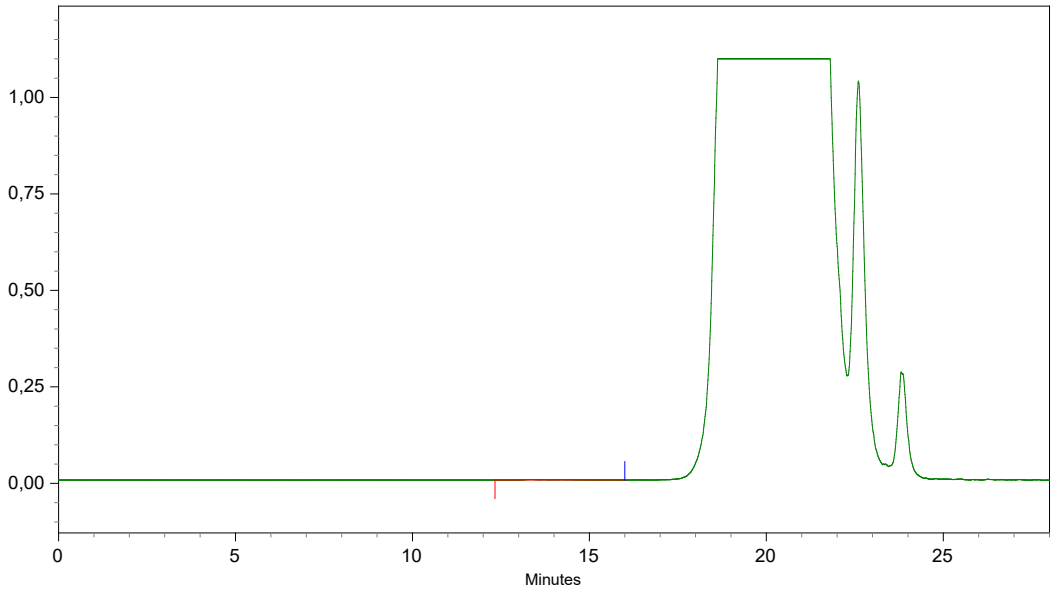


Figure B9. *HPSEC integration analysis result post-SPE of five parallel extractions of 12 mg hexane-soluble TOP 4 sample (Gain 6).*

Table B4. *HPSEC integration analysis retention time and its corresponding peak area for the post-SPE of five parallel extractions of 12 mg hexane-soluble TOP 4 sample (Gain 6).*

Peak No.	Retention time, min	Area[a]	Area Percent
1	13.49	76801	100.0
Total		**76801**	**100.0**

[a]Integration events parameters: width: 200, slope: 10, min. area: 10000 (time: 0.075 min), lock on: 16.5 min

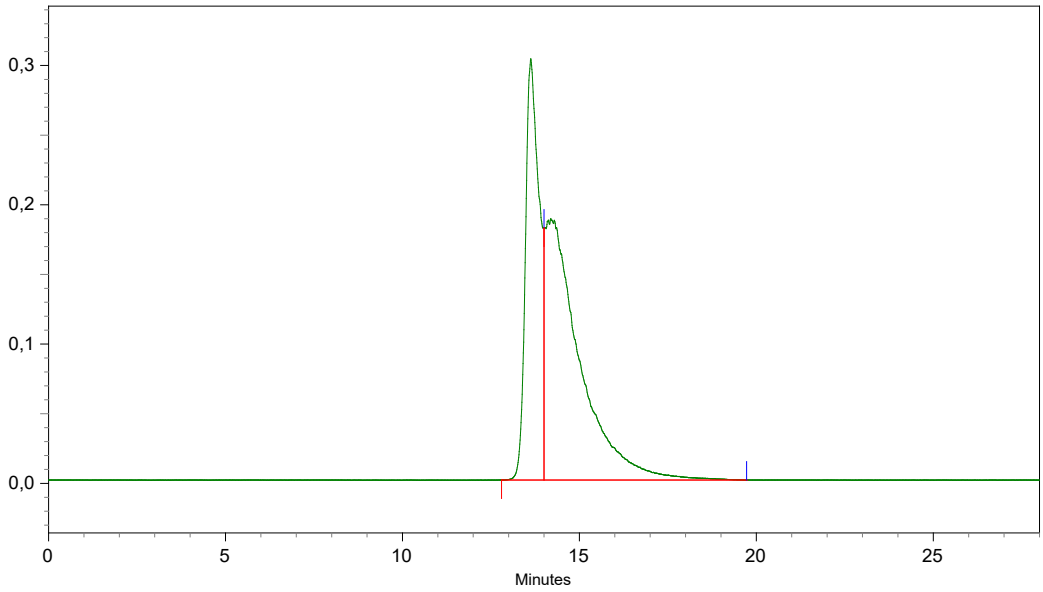


Figure B10. *HPSEC integration analysis result of the Aldrich silicone oil solution in n-hexane without SPE (Gain 3).*

Table B5. *HPSEC integration analysis retention times and their corresponding peak area for the Aldrich silicone oil solution in n-hexane without SPE (Gain 3).*

Peak No.	Retention time, min	Area[a]	Area Percent
1	13.62	8384248	39.5
2	14.11	12830608	60.5
Total		**21214857**	**100.0**

[a]Integration events parameters: width: 15, slope: 1000, min. area: 10000 (time: 0.075 min)

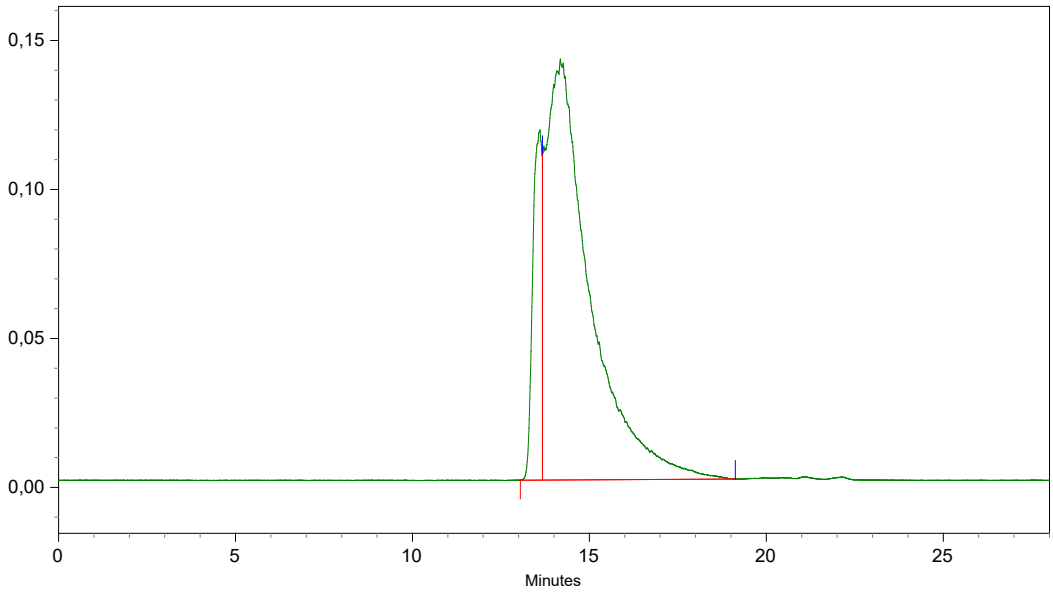


Figure B11. *HPSEC integration analysis result of eluates E7 to E9 of the Aldrich silicone oil solution in n-hexane with SPE (Gain 3).*

Table B6. *HPSEC integration analysis retention times and their corresponding peak area for eluates E7 to E9 of the Aldrich silicone oil solution in n-hexane with SPE (Gain 3).*

Peak No.	Retention time, min	Area[a]	Area Percent
1	13.58	2121550	34.9
2	14.18	12126833	85.1
Total		**14248383**	**100.0**

[a]Integration events parameters: width: 15, slope: 1000, min. area: 10000 (time: 0.075 min), lock on: 19.5 min

```
================================================================================
                                6890 GC METHOD
================================================================================


OVEN
   Initial temp:  80 'C (On)                Maximum temp:  350 'C
   Initial time:  1.00 min                  Equilibration time:  0.50 min
   Ramps:
      #  Rate  Final temp  Final time
      1  6.00      320        8.00
      2   0.0(Off)
   Post temp:  0 'C
   Post time:  0.00 min
   Run time:  49.00 min


FRONT INLET (SPLIT/SPLITLESS)           BACK INLET (UNKNOWN)
   Mode:  Splitless
   Initial temp:  300 'C (On)
   Pressure:  15.96 psi (On)
   Purge flow:  15.0 mL/min
   Purge time:  0.00 min
   Total flow:  18.2 mL/min
   Gas saver:  Off
   Gas type:  Helium


COLUMN 1                                COLUMN 2
   Capillary Column                        (not installed)
   Model Number:  Agilent 19091Z-002
   HP-1  Methyl Siloxane
   Max temperature:  325 'C
   Nominal length:  25.0 m
   Nominal diameter:  200.00 um
   Nominal film thickness:  0.11 um
   Mode:  constant flow
   Initial flow:  0.8 mL/min
   Nominal init pressure:  15.97 psi
   Average velocity:  36 cm/sec
   Inlet:  Front Inlet
   Outlet:  MSD
   Outlet pressure:  vacuum
```

Figure C1. *HP 6890 Series GC System column and oven specifications in the analysis of hexane-soluble TOP sample fractions.*

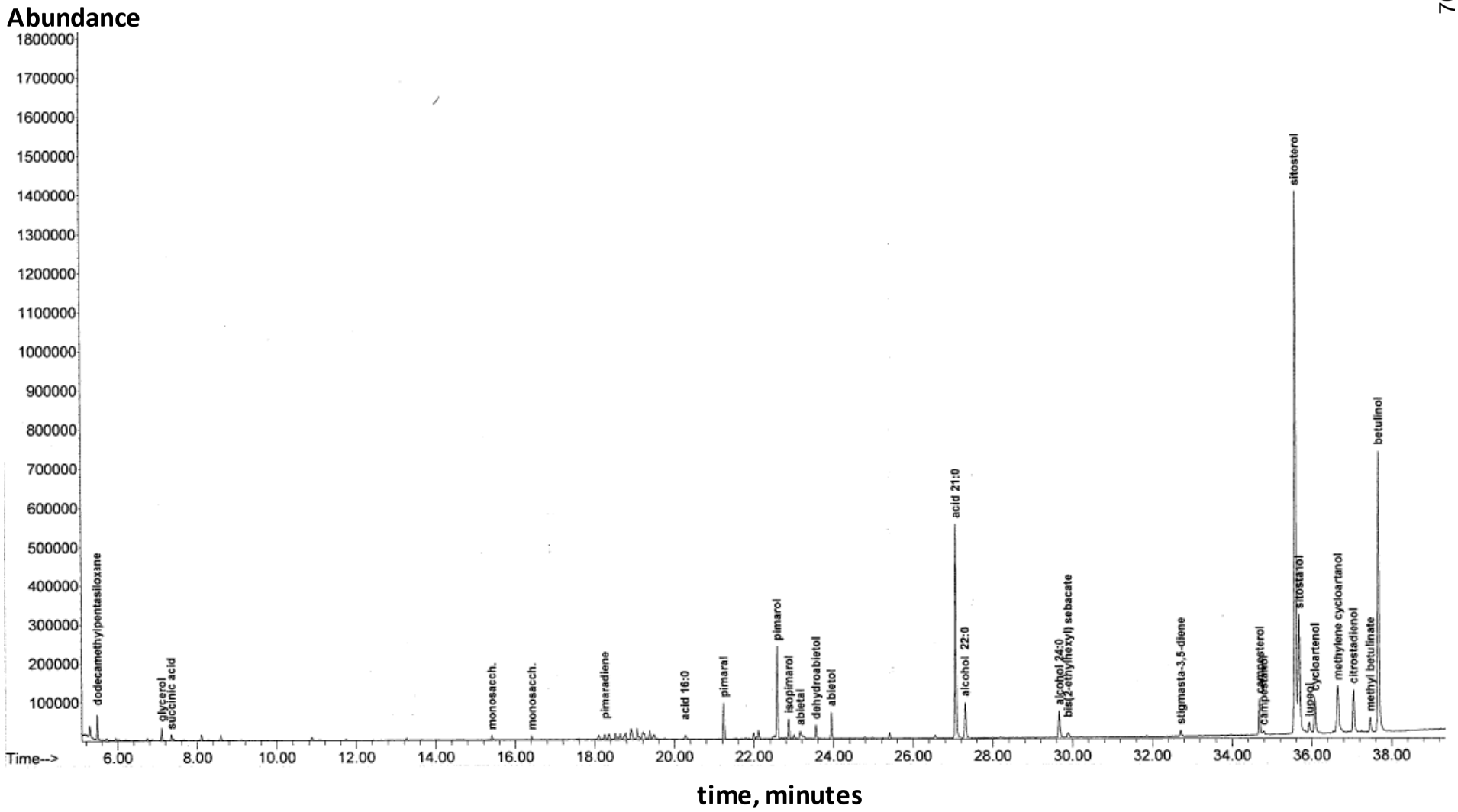


Figure C2. *GC-MS chromatogram of the TOP 4 hexane-soluble fraction hydrolysed with 2 mL of 0.5 M KOH in 90% ethanol.*

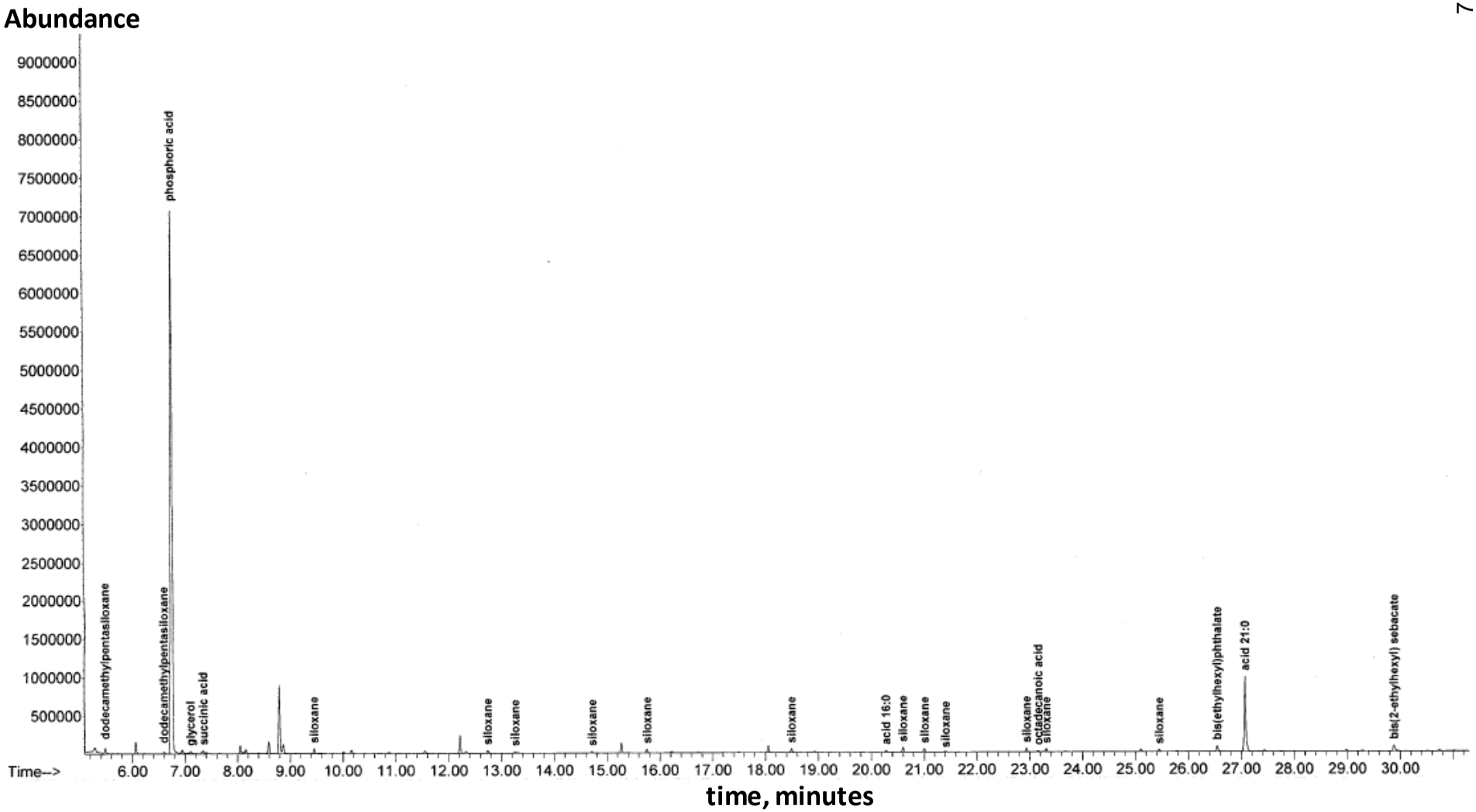


Figure C3. *GC-MS chromatogram of 1 mg/mL Aldrich silicone oil hydrolysed with 2 mL of 0.5 M KOH in 90% ethanol.*

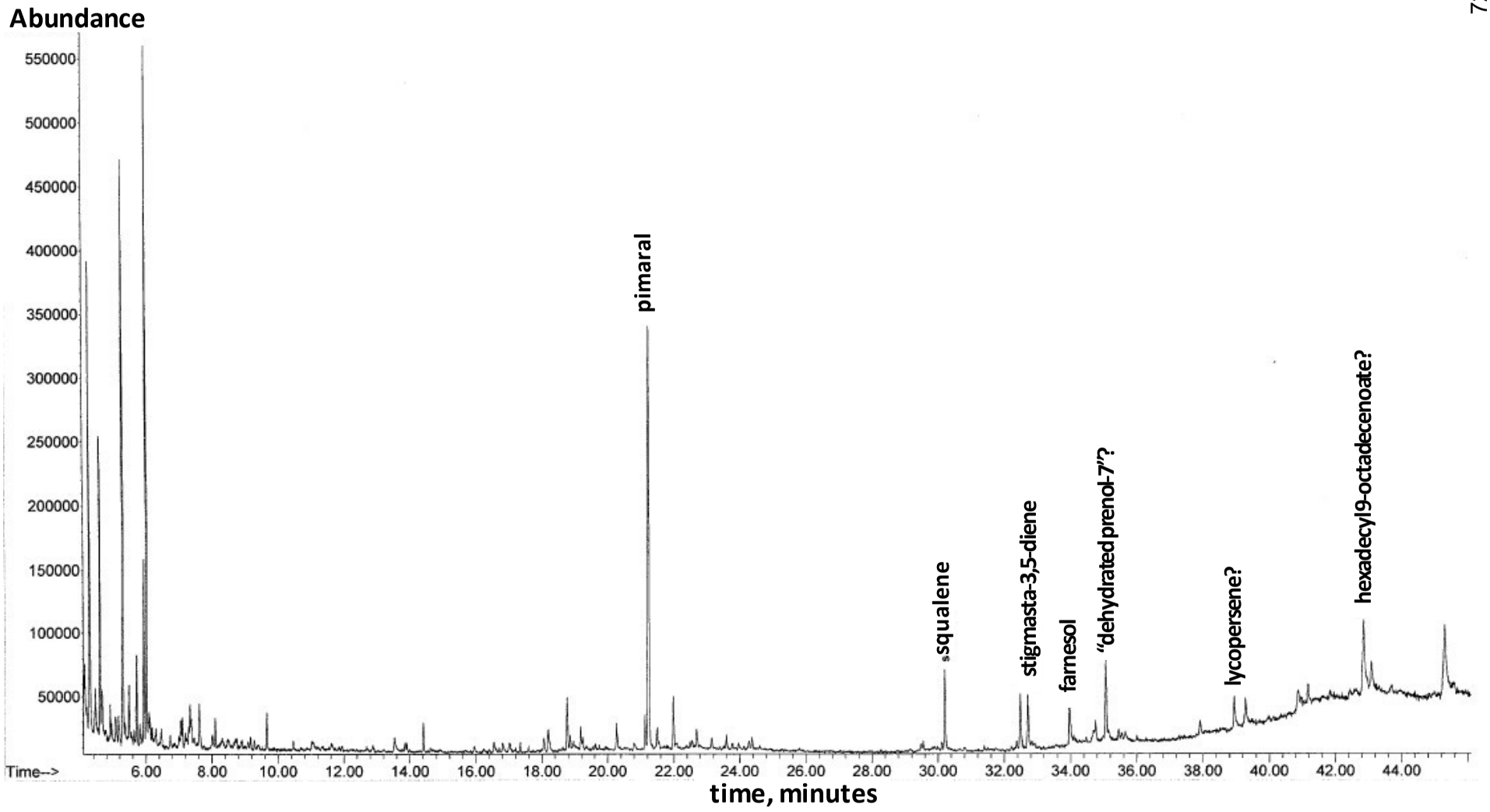


Figure C4. *GC-MS chromatogram post-SPE of 1.5 mL (~30 mg) TOP 4 hexane-soluble fraction spiked with 11% w/w Aldrich silicone oil solution in hexane (eluates E2-E3)*

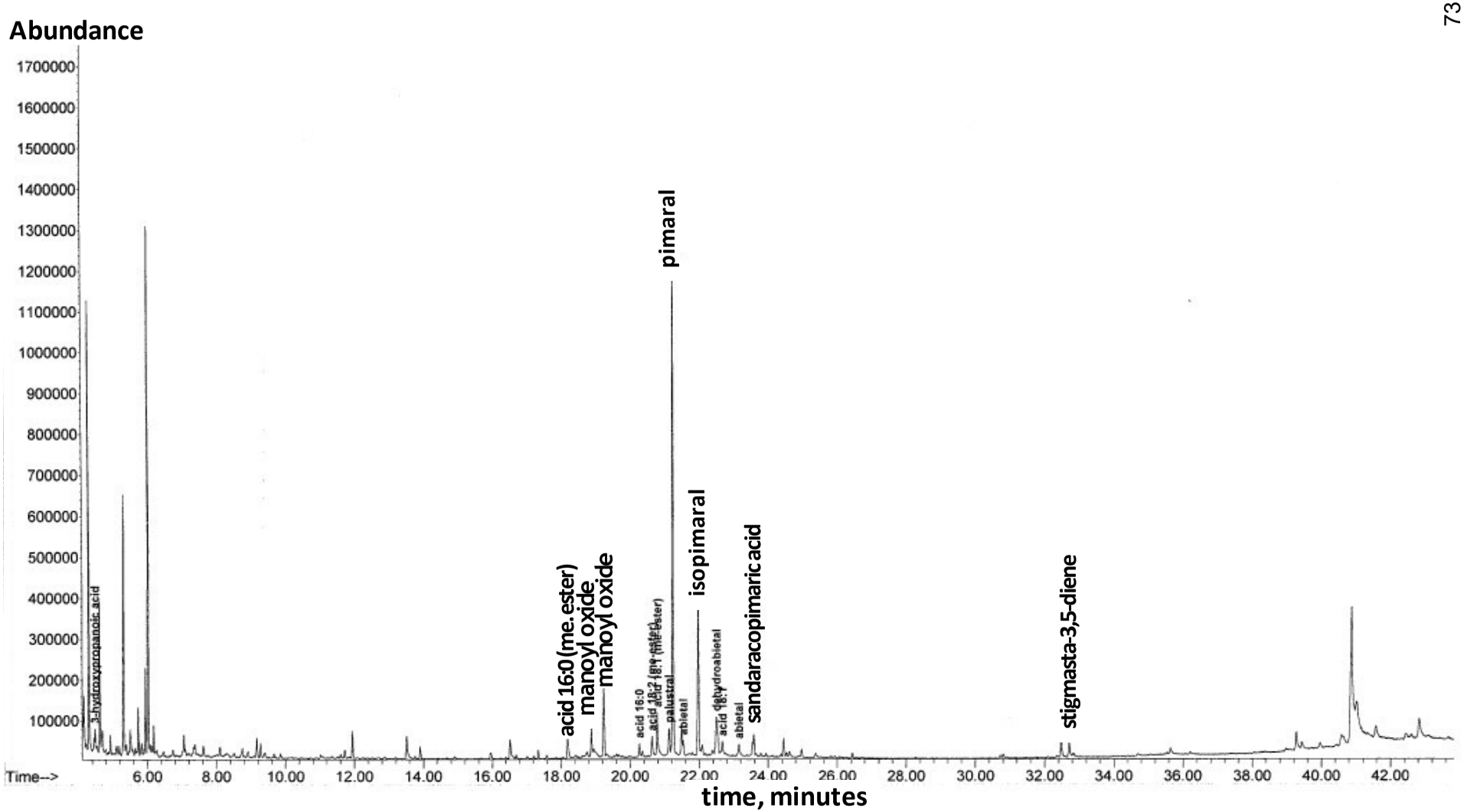


Figure C5. *GC-MS chromatogram post-SPE of 1.5 mL (~30 mg) hexane-soluble TOP 4 fraction spiked with 11% w/w Aldrich silicone oil solution in hexane (eluates E4-E5)*

Appendix D. Alkaline hydrolysis

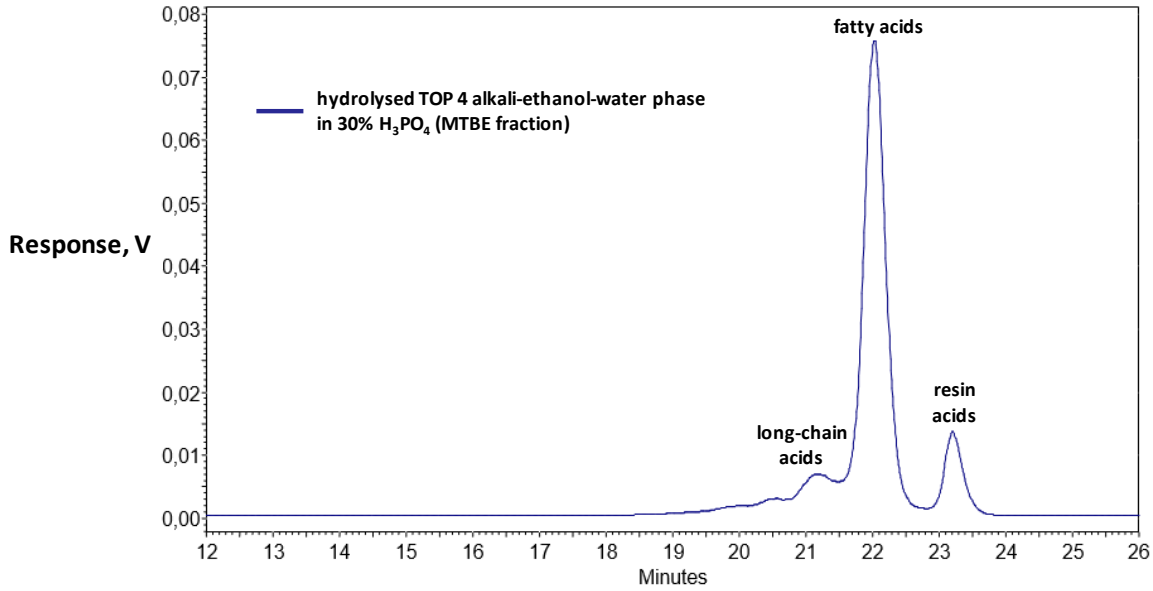


Figure D1. *HPSEC chromatogram of the MTBE-soluble fraction of the 2 mg/mL hydrolysed TOP 4 sample upon addition of 30% H_3PO_4 acid (Gain 3).*

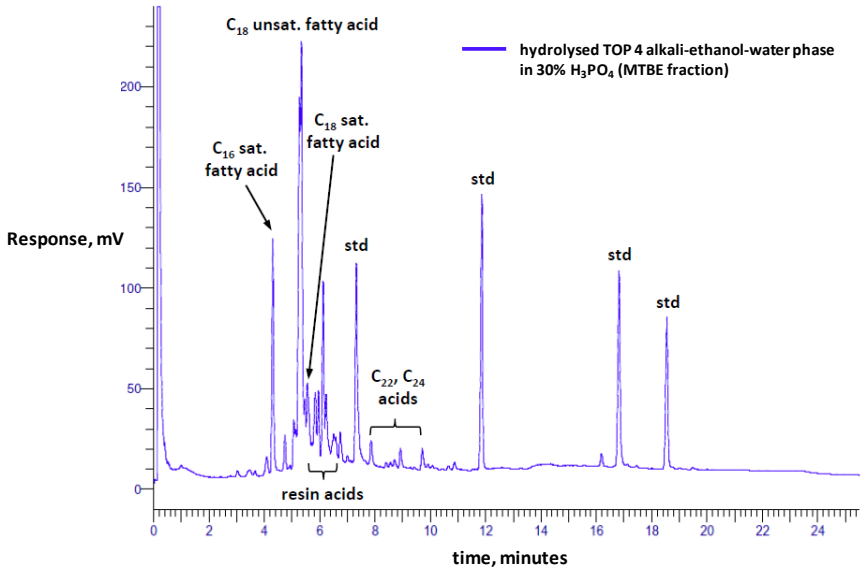
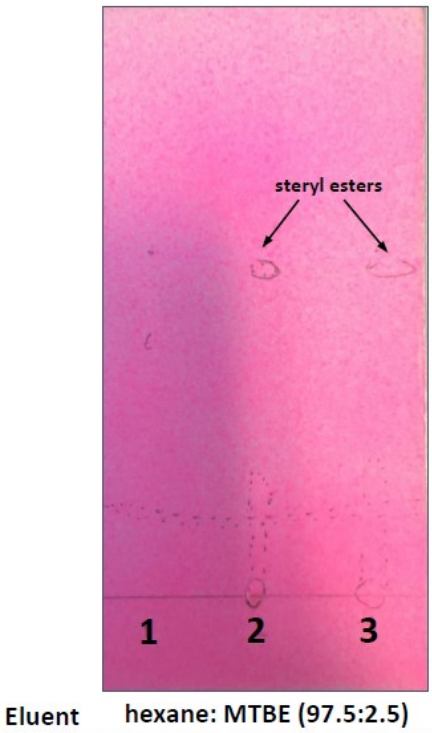


Figure D2. *Short-column GC-FID chromatogram of the MTBE-soluble fraction of the 2 mg/mL hydrolysed TOP 4 sample upon addition of 30% H_3PO_4 acid.*

Appendix E. Thin-layer chromatography



1: 50 mg/mL Aldrich silicone oil solution in n-hexane
2: TOP 4 hexane-soluble fraction
3: 32 mg/mL hydrolysed TOP 4hexane-soluble fraction

Figure E1. *TLC results of a 50 mg/mL Aldrich silicone oil solution in n-hexane (1), unhydrolysed (2), and hydrolysed (3) TOP 4 hexane-soluble fractions showing the conversion of steryl esters to sitosterol (sprayed with rhodamine B in ethanol solution).*

Appendix F. ICP-MS results

Table F1. *Si mass distribution of the hexane- and methanol-soluble fractions of the four TOP samples post-solvent extraction*

Sample	mass of sample, mg	mass of extracted hexane-soluble fraction, mg	mass of extracted methanol-soluble fraction[a], mg	Si mass in sample, µg	Si mass in the hexane-soluble fraction, µg	Si mass in the methanol-soluble fraction, µg	Si mass in the hexane-soluble fraction, %	Si mass in the methanol-soluble fraction, %	Total mass recovery, %
TOP 1	252.40	196.00	56.40	30.15	26.88	2.26	89.1	7.5	**96.6**
TOP 2	255.02	198.94	56.08	26.60	26.83	2.31	100.9	8.7	**109.5**
TOP 3	247.94	195.23	52.71	25.08	23.10	1.91	92.1	7.6	**99.7**
TOP 4	255.75	203.99	51.76	27.51	23.89	2.36	86.8	8.6	**95.4**

[a]Mass difference of the sample and the extracted hexane-soluble fraction

Table F2. *Agilent 8900 Triple Quad ICP-MS/MS technical specifications during analysis of the four TOP samples*

Instrument	Agilent 8900 ICP-MS Triple Quad Agilent SPS 4 Autosampler		
Nebulizer	MicroMist		
Lenses	x-lens w/ brass skimmer base		
Cones	Platinum sampling and skimmer cone		
Plasa Parameters	Makeup Gas	0	L/min
	Auxillary Gas	0.90	L/min
	Plasma Gas	15.0	L/min
	RF Power	1550	W
	Sample Depth	10.0	mm
	Spray Chamber temp.	2	°C
O_2 gas mode	Nebulizer gas flow	0.99	L/min
	O_2 flow	20	% in He
	Octopole bias	-5.0	V
	Omega bias	-145	V

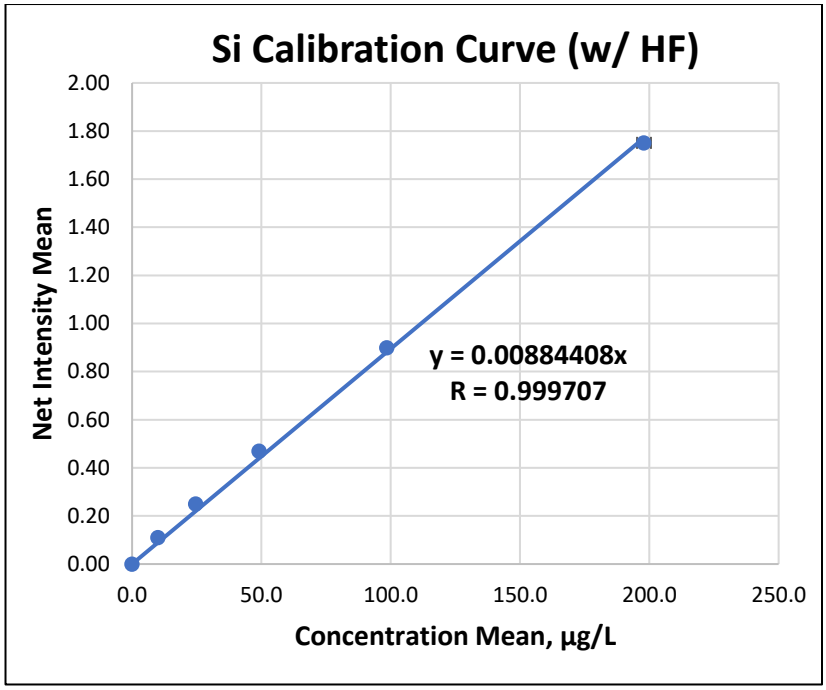


Figure F1. *ICP-MS calibration curve for 10 to 200 ppb Si standard solutions with the addition of 10 µL HF acid.*

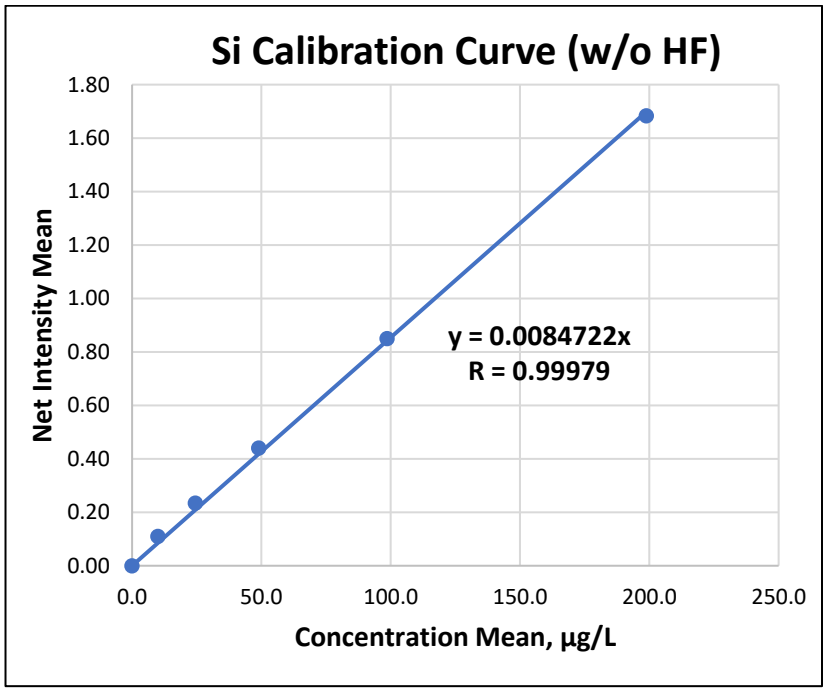


Figure F2. *ICP-MS calibration curve for 10 to 200 ppb Si standard solutions without HF acid addition.*

Table F3. *ICP-MS calibration curve tables for 10 to 200 ppb Si standard solutions with (Calibration A) and without (Calibration B) addition of HF acid.*

Calibration A (with HF, linear thru zero):

Calibrant, ppb	Conc. Mean, ppb	Standard Error, ppb	Net Intensity Mean
0	*0.000*	*0.000*	*0.000*
10	10.000	0.060	0.110
25	24.654	0.143	0.249
50	49.163	0.137	0.469
100	98.535	0.330	0.899
200	197.936	2.680	1.751

Calibration B (without HF, linear thru zero):

Calibrant, ppb	Conc. Mean, ppb	Standard Error, ppb	Net Intensity Mean
0	*0.000*	*0.000*	*0.000*
10	10.000	0.073	0.110
25	24.436	0.097	0.235
50	48.992	0.083	0.441
100	98.576	0.337	0.850
200	198.816	0.580	1.684

Stats:	w/ HF	w/o HF
slope	0.00884408	0.008472
σ_A	1.07E-04	8.68E-05
intercept	0	0
σ_B	0	0
R	**0.999707**	**0.99979**

Table F4. *ICP-MS results of the four TOP samples and two blank solutions with (Run A) and without (Run B) addition of HF acid.*

Sample	Run A (w/ HF)		Run B (w/o HF)	
	Conc. Mean, ppm	Standard Error, ppm	Conc. Mean, ppm	Standard Error, ppm
TOP 1	122.709	0.491	111.610	0.632
TOP 2	90.748	0.635	87.555	0.555
TOP 3	110.965	1.036	84.957	0.255
TOP 4	88.277	0.353	75.219	0.602
Blank 1[a]	*280.781*	0.720	3.696	0.100
Blank 2[a]	16.475	0.123	2.595	0.057

[a]units are in μg/L